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ORIGINAL ARTICLES

COMPARISON OF PROPERDIN LEVELS IN GENERAL MEDICAL AND HEMATOLOGIC PATIENTS

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The original observations (Pillemer *et al.*[22]) implicating the properdin system as an important factor in the natural defense mechanism elicited studies by numerous workers, with the result that controversy exists as to the nature of properdin (Nelson[18], Wedgewood[34]), its biologic significance (Nelson[18], Osawa and Muschel[20], Wedgewood[34]), and the adequacy of currently used assay procedures (Isliker and Linder[9], Leon[14], McNall[16]). It has been suggested that the activity attributed to properdin could be related to normal antibody (Nelson[18], Osawa and Muschel[20]). A biologic *in vivo* function of properdin has not been demonstrated (Wedgewood[34]), although *in vitro* antibacterial (Wardlaw and Pillemer[33]) and antiviral (Wedgewood, Ginsberg and Pillemer[35]) properties have been described. Studies of properdin levels in various disease processes have been inconclusive and inconsistent in that divergent results have been reported in both normal individuals (Hinz[7], Soulier and Menachee[31]) and patients with various diseases (Baltch *et al.*[1], Diomede-Fresa and Fumarola[3], Hinz[7], Hunter and Hill[8], Laurell[12], Myerburg[17], Newcomer *et al.*[19], Soulier and Menachee[31], Southam and Pillemer[32]). In part, this variation may be attributed to differences in assay procedures and the small sampling groups in most of the studies.

Previous studies from this laboratory showed that the mean properdin titer in 105 splenectomized subjects was definitely lower than that of 113 normal persons (Carlisle and Saslaw[2]), and was associated statistically with absence of the spleen rather than with the underlying pathologic process. This

suggested further investigation of the effect of disease *per se* on properdin level.

The present report describes a study of properdin assay results in 508 randomly selected hematology and general medical clinic patients. A concurrent study of antibody production in hematologic patients (Saslaw, Carlisle and Bouroncle[29]) provided an opportunity to compare properdin level and ability to produce antibodies after tularemia vaccination in 87 of the hematologic patients.

Materials and Methods. Subjects were selected at random, without prior knowledge of specific diagnosis, from patients visiting the general medical and hematology clinics at University Hospital over a period of 3 months. Normal controls were selected from presumably healthy volunteers. Serum properdin levels were determined by the Pillemer zymosan assay (Pillemer *et al.*[23]). Methods used in controlling sensitivity and reliability of the assay were described in our earlier report (Carlisle and Saslaw[2]).

Results. Serum properdin levels observed in 256 hematology clinic patients, representing 21 different diagnostic categories, are presented in Table 1. The most notable finding in these patients was the wide range of values seen in most of the disease category groups. None of the individual disease entities was characterized by a complete lack of properdin, and in no case was it obvious that levels were different from those seen in the group of 113 normal subjects. The mean properdin titers of each of the various disease category groups containing sufficient subjects for analysis were not significantly different from each other or from the mean

TABLE 1.—PROPERDIN LEVELS IN 256 HEMATOLOGY CLINIC PATIENTS

Diagnostic Group	*No. of Cases*	*Properdin Units* 0–1	1½–2	3–4	6–8	12–16	*Mean*
Lymphoma	76	13	8	14	19	22	7.18*
Chronic Lymphatic Leukemia	50	10	12	9	11	8	5.28†
Chronic Myeloid Leukemia	30	16	1	3	4	6	4.20†
Pernicious Anemia	21	7	1	2	8	3	5.43†
Idiopathic Thrombocytopenic Purpura	12	5	3	1	2	1	3.68
Polycythemia Rubra Vera	10		3	2	3	2	6.95
Iron Deficiency Anemia	10	3	1	3	1	2	5.00
Monoblastic Leukemia	9	2		3	3	1	5.67
Hypoplastic Anemia	6	1		4		1	5.33
Hemolytic Anemia	6	4		1		1	3.50
Myelofibrosis	4	4					0.00
Splenic Neutropenia	4		1		1	2	8.50
Leukopenia	3	1		1	1		4.00
Sickle Cell Anemia	3			1	2		6.67
Reticulum Cell Sarcoma	2			1	1		6.00
Multiple Myeloma	2	1				1	8.00
Hemochromatosis	2	1			1		3.00
Clotting Defect	3			1	1	1	8.67
Sarcoid with Anemia	1	1					0.00
Macrocytic Anemia	1	1					0.00
Porphyria	1				1		8.00
	256	70	30	46	59	51	5.67†
Normal Subjects	113	13	9	21	55	15	6.35

*Distribution of properdin values significantly different from normals, 1% level.
†Distribution of properdin values significantly different from normals, 5% level.

(6.35 units) of the group of normal individuals (Table 1). The mean properdin titer (5.67 units) of the total group of 256 hematologic patients also was not significantly different from the mean of the normals.

There was, however, a statistically significant difference in the *distribution* of properdin values in certain of the disease categories as compared to the control subjects. For example, 22 of 76 (29%) lymphoma patients (Fig. 1) and only 15 of 113 (13%) normal individuals had 12 to 16 units of properdin, a range that can be considered high or high normal. The large number of high properdin levels in lymphoma patients was partially offset by a high incidence of low values (17%) so that the mean, 7.18, was not significantly different from the mean (6.35) of the normals. Similarly, a wide distribution of properdin values was observed in 50 chronic lymphatic leukemia, 30 chronic myeloid leukemia, and 21 pernicious anemia patients. These four groups accounted for about two-thirds of the hematologic patients studied. This was reflected in the significantly wider distribution noted in the total group of 256 hematology clinic patients than in normal controls.

Properdin levels in 252 general medical clinic patients (Table 2) followed a pattern similar to that seen in patients with hematologic disorders. The mean properdin titers of the various disease category groups were not significantly different from the mean of the normal subjects (6.35), except that the mean was significantly lower (4.40) in 75 patients with cardiovascular disease. Seven of the groups (Table 2) exhibited a distribution of properdin values different from the normals, in that a greater incidence of both higher and lower values was observed as with certain hematologic patient groups noted above. This group of 252 medical clinic patients was made up of individuals with about 75 different specific diagnoses. The classification of these subjects presented in Table 2 was one of three, which was made and analyzed in an attempt to show an association between properdin level and specific

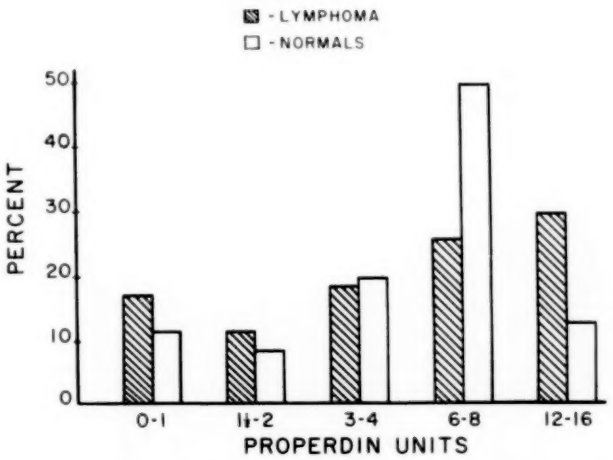

Fig. 1.—Distribution of properdin levels in 76 lymphoma patients and 113 normal subjects.

disease entity or disease group. The two analyses not included in the present report did not provide any significant facts other than those discussed in connection with Table 2.

An analysis of the total group of 508 medical and hematologic clinic patients by the multiple regression method (Snedecor[30]) revealed no correlation between properdin titer and sex or skin color. Levels were significantly lower, however, in 49 subjects over 70 years of age. These patients were distributed rather uniformly throughout the various groups and it was shown that age was not the determining factor in the observed low values in the patients with cardiovascular disease. There was no correlation between properdin level and type of treatment in the 256 hematologic patients. For example, 51 subjects had received recent Roentgen-ray therapy, but it was shown, statistically, that properdin titer was not associated with this type of treatment. Certain of the larger disease category groups were analyzed separately to determine if a correlation existed between the properdin level and the severity or duration of the illness, and presence or absence of anemia. No significant correlations were detected.

As stated previously, the *distribution* of properdin values in both the medical and hematologic patients was different from that observed in normal individuals. The distribution in these three groups is compared with that seen in 105 splenectomized subjects

TABLE 2.—PROPERDIN LEVELS IN 252 MEDICAL CLINIC PATIENTS

Diagnostic Group	*No. of Cases*	*Properdin Units*					*Mean*
		0–1	*1½–2*	*3–4*	*6–8*	*12–16*	
Arteriosclerotic & Hypertensive Cardiovascular Disease	63	22	11	12	7	11	4.57*
Other Cardiovascular Disease	12	6		4		2	3.50
Total Cardiovascular Disease	75	28	11	16	7	13	4.40*
Respiratory	13	4		1	4	4	7.54*
Gastrointestinal	33	10	5	5	7	6	5.50*
Genitourinary	8	3	1	1	3		3.88
Gynecologic	11	2		5	3	1	5.09
Neurologic	5			3	1	1	6.40
Diabetes	7		1	1	1	4	10.57
Other Endo-Metabolism	8	4	1	1	1	1	3.19
Total Endo-Metabolism	15	4	2	2	2	5	6.63*
Degenerative Arthritis	17	4	3	4	2	4	6.03*
Other Musculo-skeletal	19	7	2	3	2	5	5.68*
Total Musculo-skeletal	36	11	5	7	4	9	5.85*
Allergic	3	1				2	9.33
Head, Ear, Eye, Nose & Throat	6	2	1		2	1	5.67
Carcinoma	9	3	3	1	1	1	3.33
Functional	36	10	6	6	7	7	5.08†
Miscellaneous	2	1			1		4.00
	252	79	34	47	42	50	5.24†
Normal Subjects	113	13	9	21	55	15	6.35

*Distribution of properdin values significantly different from normals, 1% level.
†Distribution of properdin values significantly different from normals, 5% level.

(Carlisle and Saslaw[2]) in Fig. 2. It can be seen that there is little difference between the medical and hematologic patients from the standpoint of distribution of properdin titers, but both groups are different in this respect from normal subjects and decidedly different from splenectomized individuals. The incidence of high values in the splenectomized group was very low, and except for this characteristic, properdin titers in the splenectomized individuals were much like those of the medical and hematologic patients.

Eighty-seven of the nonsplenectomized hematologic patients in this series also participated in a concurrent study of the ability of patients of this type to produce antibodies after a single subcutaneous injection of Foshay tularemia vaccine (Saslaw *et al.*[28,29]). Table 3, which presents properdin assay results in 35 patients who produced antibodies and in 52 who did not, shows that there was no correlation between properdin level and ability to exhibit antibodies in these patients. The mean properdin titer of the group which showed antibodies was not significantly different from that of the group which did not. There also was no significant difference in the distribution of properdin values observed in the two groups of patients. These results are in agreement with our previous observation[2] of the lack of cor-

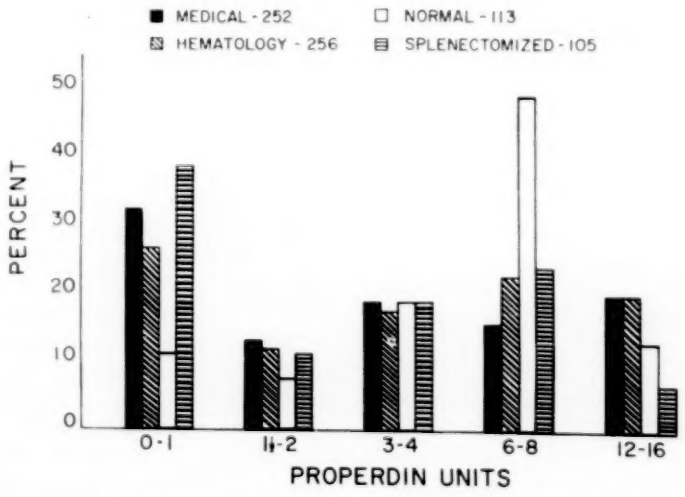

Fig. 2.—Properdin levels in medical and hematology clinic patients and in splenectomized and normal individuals.

TABLE 3.—COMPARISON OF PROPERDIN LEVEL AND ABILITY TO PRODUCE ANTIBODIES AFTER TULAREMIA VACCINATION IN 87 HEMATOLOGIC PATIENTS

No. of Subjects	*Antibodies Produced*	*Properdin Units*								
		0	*1*	*2*	*4*	*6*	*8*	*12*	*16*	*Mean*
35	+	9	1		11	2	7	2	3	5.29
52	−	8	3	9	11	2	10	2	7	5.67
87	Total	17	4	9	22	4	17	4	10	5.49

relation between properdin level and ability to produce antibodies in splenectomized individuals.

Discussion. The most notable finding obtained from this study of properdin levels in 508 medical and hematologic patients was the wide range of values observed in most of the disease categories. An abnormally high incidence of high values was seen in many of the groups, but the means were not significantly different from the mean of the normals since the incidence of low values was also high. The *distribution* of properdin titers in groups of patients, and particularly the occurrence of high values, has not been emphasized in the literature. Studies by other workers (Diomede-Fresa and Fumarola[3], Eyquem and Tullis[4], Fumarola[5], Halde *et al.*[6], Hinz[7], Hunter and Hill[8], Laurell[12], Laurell and Grubb[13], Lowbury and Ricketts[15], Myerburg[17], Newcomer *et al.*[19], Rottino and Levy[26], Rottino, Levy and Conte[27], Soulier and Menachee[31], Southam and Pillemer[32]) have been concerned primarily with the occurrence of low values, although Rottino and Levy[26] and Fumarola[5] observed high values in patients with Hodgkin's disease and lymphosarcoma, respectively.

The theoretical basis for the occurrence of both low and high properdin values has been suggested by animal experimentation (Landy and Pillemer[10,11], Pillemer, Landy and Shear[24], Pillemer and Ross[25]) in which it has been shown that certain complex substances containing high-molecular-weight polysaccharides (for example, zymosan, bacterial cell walls, cell walls of human erythrocytes, and substances from normal and neoplastic tissue) could either depress or elevate properdin levels *in vivo* depending upon the amount injected. From these studies, Pillemer[21] suggested that "an elevation in titer may be due to liberation of small amounts of bacterial or tissue products, while a fall in properdin titer may accompany liberation of large amounts of these substances." On purely theoretical grounds, it might, therefore, be said that properdin level is an indicator of the degree of tissue breakdown and, as such, may be of prognostic importance. However, in the present study, there was apparently no correlation between properdin titer and severity or duration of illness. Rottino, Levy and Conte[27] also noted no correlation, in 109 cancer patients, between properdin level and presence or absence of metastasis or anemia, the degree of advancement, or site and type of the malignant process. On the other hand, Diomede-Fresa and Fumarola[3] observed that properdin levels were lower in patients with advanced cancer and that properdin titer was, in general, associated with the stage of the malignant process.

Precise comparison of human properdin levels determined in various laboratories is impractical since different assays (Isliker and Linder[9], McNall[16], Pillemer *et al.*[23]) have been used and since the level of sensitivity of the test has not been the same from study to study, as represented by the fact that widely divergent results have been obtained in groups of normal subjects. For example, Hinz[7] reported that all normal individuals studied had two or more units of properdin, whereas Soulier and Menachee[31] found that 26% of 115 normal subjects had less than one unit. Other workers have reported intermediate values. In our series, 7% of 113 healthy, normal individuals were without detectable properdin.

Certain nonstatistical comparisons can be made, however, between our results and those of other workers. Our observations of low values in cardiovascular disease patients and of

both low and high values in patients with Hodgkin's disease are similar to those of Rottino *et al.*[26,27]. Properdin levels in leukemic patients have been reported to be low (Baltch *et al.*[1]), and high (Rottino and Levy[26]). In our patients, both low and high values were seen. Low titers have been observed by others in patients with carcinoma (Fumarola[5], Myerburg[17], Rottino and Levy[26], Rottino, Levy and Conte[27], Southam and Pillemer[32]), rheumatoid arthritis (Laurell and Grubb[13]), pyelonephritis (Hinz[7]), diabetes accompanied by cutaneous or systemic bacterial infections (Eyquem and Tullis[4]), coccidioidomycosis (Halde *et al.*[6]), and severe thermal burns (Lowbury and Ricketts[15]). Our observation of low levels in patients over 70 years of age is in agreement with those of Newcomer *et al.*[19] and Fumarola[5].

These studies suggest that employing the mean properdin titer as the sole measure of comparison between normal and various disease category subjects may be misleading. This is particularly so when sample groups are small. The range or distribution of values may readily affect the mean, and variation from very high to very low properdin levels may be encountered in various disease categories.

Splenectomized subjects were different from medical and hematologic patients, with intact spleens, in that the high values noted in the two latter groups were not observed. The significance of this finding in relation to the basis for low properdin titers in the splenectomized subjects is not known at present.

Summary. Studies in this laboratory have shown no significant differences in group mean properdin levels between 113 normal individuals, 256 hematology and 252 general medical clinic patients. However, mean properdin titers were definitely lower in splenectomized subjects and in patients with cardiovascular disease. In large groups of patients, it was apparent that the range of variation was great. Thus, although the mean titers in the hematologic group were similar, considerably greater difference in incidence of both high and low titers in patients with lymphomas, chronic lymphatic and chronic myeloid leukemia and in pernicious anemia was observed. Similarly in general medical patients, this wide distribution was particularly evident in those with cardiovascular, respiratory, gastrointestinal, metabolic, musculoskeletal and functional complaints. These observations may account, in part, for the discrepancy in properdin levels reported from various studies in which smaller sampling groups have been used. The significance or utility of properdin levels in health or disease is not clear at this time.

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SUMMARIO IN INTERLINGUA

Comparation del Nivellos de Properdina in Patientes Generalmente Medical e in Patientes Hematologic

Studios a iste laboratorio ha monstrate nulle significative differentias in le valores medie del nivellos de properdina inter un gruppo de 113 subjectos normal, un gruppo de 256 patientes hematologic, e un gruppo de 252 patientes clinic generalmente medical. Tamen, le titros medie de properdina esseva definitemente plus basse in subjectos splenectomisate e in patientes con morbo cardiovascular. In grande gruppos de patientes il esseva apparente que le region del variationes esseva extense. Assi, ben que le titros medie in le gruppo hematologic esseva simile, considerabilemente plus pronunciate differentias in le incidentia de titros alte e de titros basse esseva observate in patientes con lymphomas, con chronic leucemia lymphatic o myeloide, e con anemia perniciose. Similemente in le gruppo del patientes generalmente medical, ille large distribution esseva particularmente evidente in patientes con disordines cardiovascular, respiratori, gastrointestinal, metabolic, musculo-skeletic, e functional. Iste observationes explica forsan—al minus in parte—le discrepantia inter le nivellos de properdina reportate variemente a base de studios con plus micre gruppos-specimen. Le signification o le utilitate del nivellos de properdina in sanitate o in morbo non es clar a iste tempore.

STUDIES ON STAPHYLOCOCCI OF PHAGE TYPE "80/81"

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Since the isolation of phage 80 (Rountree and Freeman[9]) and 81 (Bynoe, Elder and Comtois[5]), staphylococci lysed by one or both of these phages have been frequently observed in almost every country where phage typing is used. Type "80/81" is now the most prevalent type among staphylococci in many hospitals (Blair and Carr[2], Williams[15]) and also appears to be thoroughly disseminated in the community, at least as judged from their occurrence in lesions among outpatients coming to this hospital (Wallmark and Finland[14]). Schreck[10] in a study of 1060 strains isolated from hospital-acquired infections during the 4 years 1956-1959 found 55% of them to be of this type. Nahmias *et al.*[8] found that this type accounted for about 60% of strains in some of their material. Among strains isolated in 1959-1960 at this hospital, 35% of those from hospitalized patients and 38% of those from outpatients, typed as "80/81" (Wallmark and Finland[14]).

Bynoe *et al.*[5], comparing their phage 81 with phage 80, found that nearly all strains lysed by 81 were also lysed by 80. On the other hand, Nahmias and coworkers[8] observed several strains that were lysed by 80 or 81, but not by both. It is now generally agreed that these two phages are closely related, but not identical. They are of different serological types, 80 being of group B and 81 of group A.† Although there are indications that strains typing as 80, 81, and 80/81 (or 52/52A/80, and others) might be different, most workers prefer to consider such strains as identical, and the designation "80/81" is now commonly used (Blair and Bynoe[1], Blair and Carr[3], Williams[15]). However, the data presented by Nahmias *et al.*[8] indicated that type 80/81 which they termed "80/81 complex" consists of a group of distinct, though related, types and subtypes.

In a number of reports from this country during the last few years, a high incidence of hospital infections caused by staphylococci typed as 52/42B/81 has been observed (Blair and Carr[2]). At this hospital, 61 (34%) of 180 cultures isolated in 1955 were re-

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†Noted by P. M. Rountree (J. Gen. Microbiol., **20,** 620, 1959).

ferred to this type (Finland, Jones and Bennett[6]). Blair and Bynoe[1], in a comparative study, found that the phages 52 and 42B used in this country had mutated and had acquired a wider host range. They suggested that strains typed as 52/42B/81 should be designated as 80/81.

The purpose of the present study was twofold: 1) to compare the variants of 52 and 42B with the present set of phages, including the original 52 and 42B, and, 2) to determine whether type "80/81" might be separated into distinct types. For the latter purposes, 29 or those of Group III, were not included; such strains were regarded as clearly different. Also included for comparison are 25 strains lysed only by the related phage KS6 (Wallmark[12]).

The phage typing method was identical with the one previously used (Wallmark and Finland[14]). Most of the strains were also tested with those phages at 100 × RTD. Tests for lysogenicity were done with the cross culture method of Fisk[7], using drops of untreated broth cultures. The susceptibility of the strains to antibiotics was tested by means of the inocula replicating apparatus of Steers, Foltz and Graves[11], using two-fold dilutions of the antibiotics in agar and a 1:10 dilution of culture, which provided an inoculum of about 10^6 organisms.

TABLE 1.—LYSIS OF TYPE "80/81" STAPHYLOCOCCI BY PHAGES 52 AND 42B AND THEIR RESPECTIVE VARIANTS, 52v AND 42Bv

Pattern with Routine Phages	*Number of Strains Tested*	*Patterns with Phages 52, 52v, 42B and 42Bv*					
		52v/42Bv	*52v*	*42Bv*	*52v/42B or 52v/42Bv/42B*	*42B or 42B/42Bv*	*Negative*
80/81/KS6	132	95	5	15	7	4	6
80	10		9				1
80/81	18	11	6				1
80/KS6	21		10				11
Total	181	106	30	15	7	4	19

a number of strains with this and similar patterns were tested further for their sensitivity to the conventional phages at 1000 times the routine test dilution (RTD) and to a few other, old and new, related phages. The lysogenic properties of a number of these strains were also studied. In addition, all of the strains were tested for susceptibility to the antibiotics that are most widely used.

Materials and Methods. Only strains of coagulase positive staphylococci were included in this study. These were obtained from purulent lesions, sputum, blood, and others, most of them from patients admitted to the hospital, as part of a study reported elsewhere (Wallmark and Finland[14]). A total of 380 strains, all lysed by phages 80 or 81, or both, were included; a few of them were also lysed by phages 52 and 52A. Lytic patterns of 80/81 with other phages; for example,

Results. THE RELATIONSHIP OF THE VARIANTS OF PHAGES 52 AND 42B TO THE PHAGES 52, 42B, 80, 81 AND KS6 IN CURRENT USE. Tests were done with 181 strains that typed as 80/81/KS6 or were lysed by only one or two of these phages. The results are shown in Table 1. Of these 181 strains, 143 were lysed by the 52 variant (52v), 132 by 42B variant (42Bv), 11 by the original 42B, none by the original 52, and 19 strains (10.5%) were resistant to all 4 of these phages. These results confirm the conclusions reached by Blair and Bynoe[1] on the altered host range of phages 52v and 42 Bv and on their similarity to phages 80 and 81. The results also confirm the close relationship between the previous pattern 52/42B/81 and the present "80/81".

PHAGE PATTERNS WITHIN THE "80/81 GROUP." In the course of the study of the phage types of staphylococci isolated at Boston City Hospital during five seasons in the period 1950-1960, a total of 1550 was investigated (Wallmark and Finland[14]); 405 of these strains were classified as "group 80/81;" that is, they were lysed at RTD or in some instances only by 1000 × RTD of one or more of the phages 52, 52A, 80, 81 and KS6. Nine different patterns were observed; the frequency with which these patterns were encountered in the different years is shown in Table 2. Very few strains referred to this group were observed in 1950-1952, but in 1955 and thereafter they represented 37% to 41% of all strains. Among these strains, 80/81/KS6 was the predominant pattern in each season, but 80/81 and 80/KS6 showed a clear increase in 1959-1960.

TABLE 2.—STRAINS OF THE "80/81 GROUP" OF PHAGES IDENTIFIED DURING 5 SEASONS FROM 1950 TO 1960 AT BOSTON CITY HOSPITAL

Phage Pattern	*Strains from Hospitalized Patients*					*Outpatient Strains*		*Total*
	1950	*1951–52*	*1955*	*1958*	*1959–60*	*1958*	*1959–60*	
80/81/KS6	—	1	71	37	115	16	43	283
52/52A/80	—	—	2	1	5	2	1	11
52/52A/80/81	—	1	—	9	6	1	—	17
80	1	—	—	1	5	—	1	8
80/81	—	—	1	—	13	1	4	19
80/KS6	—	—	2	—	20	4	7	33
*81**	—	—	—	—	3	—	2	5
*81/KS6**	—	—	—	—	3	—	1	4
KS6	—	8	2	5	8	—	2	25
Total	1	10	78	53	178	24	61	405
% of all strains	3.	2.1	37.7	41.0	36.9	41.	39.	26.2

*Lysed only by 1000 × RTD.

A large number of these strains were also tested with the phages at 1000 × RTD. Among 100 strains of 80/81/KS6 tested, 91 showed the same pattern with the higher phage concentration, but 9 were lysed by 52 and 52A in addition. All 16 strains of 80/81 tested in the same manner showed the same pattern in 1000 × RTD, whereas the 5 type 80 strains tested were all also lysed by 52, but not by 81, in 1000 × RTD. The few strains of 81 and 81/KS6 were lysed by these phages and by no others, and only at 1000 × RTD. Of the 26 strains of 80/KS6, 23 gave the identical pattern with the higher concentration; 3 were also lysed by 52 and 52A, but none by 81. All 9 strains of 52/52A/80/81 tested showed the identical pattern when 1000 × RTD was used.

During the search for new phages suitable for typing the apparently increasing numbers of nontypable strains, two new phages, provisionally designated as F and G, and similar to the previous 80 and 81, were isolated (Wallmark and Finland[13]). These two phages were used to test 116 of the strains that gave various patterns that were included in the "80/81 group." Most of these strains were also lysed by these phages and no further significant information was obtained.

SUSCEPTIBILITY TO ANTIBIOTICS. The

TABLE 3.—ANTIBIOTIC RESISTANCE OF 185 STRAINS OF STAPHYLOCOCCUS AUREUS LYSED BY A VARIETY OF PHAGE PATTERNS WITHIN "GROUP 80/81" (BOSTON CITY HOSPITAL 1959–60)

Phage Pattern	*Number of Strains Tested*	*Number Resistant to:*					
		P	*Sm*	*T*	*C*	*E*	*N*
80/81/KS6	105	103	77	69	7	26	5
52/52A/80	6	3	2	1	0	0	0
52/52A/80/81	6	6	5	3	0	2	2
80	6	4	4	2	0	0	0
80/81	17	17	16	14	0	1	1
80/KS6	26	26	15	15	1	9	8
81	5	2	0	0	0	0	0
81/KS6	4	0	0	0	0	0	0
KS6	10	9	6	5	0	3	2
Total	185	170	125	109	8	41	18

P = penicillin; Sm = streptomycin; T = tetracycline; C = chloramphenicol; E = erythromycin; N = novobiocin.
"Resistant" strains were not inhibited by the following concentrations (in μg/ml.): P, 0.8; Sm, 12.5; T, 12.5; C, 25; E, 1.6; and N, 1.6 (see Wallmark and Finland[14]).

sensitivity of strains of the different types within the "80/81 group" to the 6 most commonly used antibiotics is summarized in Table 3, which lists the number of strains resistant to certain concentrations of each antibiotic.* Of the strains typing as 80/81/KS6, only those from hospitalized patients are included, whereas those of the other types include both hospital and outpatient strains. Generally, in each phage type there was a variety of antibiotic patterns (antibiograms). The majority of strains were resistant to penicillin, streptomycin and tetracycline, about one-fourth to erythromycin, but only a few to novobiocin and chloramphenicol.

The 105 strains typing as 80/81/KS6 and isolated from patients admitted to the hospital could be separated into no less than 14 "antibiotic types," as seen in Table 4. These were widely disseminated in the hospital without significant predominance of any one antibiotic pattern in any single ward.

SENSITIVITY TO LYSOGENIC PHAGES. The wide variation in the antibiotic sensitivity among strains with identical or closely similar phage patterns raises the question whether such strains should be regarded as identical or not. A study of the lysogenic properties of a limited number of strains by means of a cross-culture technique was

TABLE 4.—PATTERNS OF RESISTANCE AMONG 105 STRAINS OF PHAGE PATTERN 80/81/KS6 ISOLATED AT BOSTON CITY HOSPITAL 1959–60

*Antibiogram**						*Number of Strains*	*Number of Wards*
P	*Sm*	*T*	*C*	*E*	*N*		
R	R	R	S	S	S	41	24
R	S	S	S	S	S	26	18
R	R	R	S	R	S	17	11
R	R	S	S	S	S	6	5
R	R	R	R	R	S	4	2
R	R	R	S	R	R	3	3
8 other patterns						1 each	1 each

*R = resistant; S = sensitive; for definition of "resistant," see footnote to Table 3.

*The selection of these particular concentrations is discussed by Wallmark and Finland[14].

therefore undertaken. The lytic reactions observed in cross cultures of 12 strains typing as 80/81/KS6, 80/KS6, 80/81 and 80 and showing a variety of antibiotic sensitivity patterns are shown in Table 5.

The three strains 80/81/KS6 were not lysed by each other and they were lysed by the same 3 other strains and not by any others. They also had similar antibiograms and thus seemed to be identical. Each of the three 80/KS6 strains were lysed by both the others of that type as well as by several strains with different patterns and thus are not identical. They also had different

TABLE 5.—LYSIS IN CROSS CULTURES OF 4 GROUPS OF "80/81" STRAINS WITH DIFFERENT PHAGE PATTERNS

Strain	Phage Pattern	Strain												Antibiogram*					
		1	2	3	4	5	6	7	8	9	10	11	12	P	Sm	T	C	E	N
1	80/81/KS6				+		+						+	R	R	R	S	S	S
2	80/81/KS6				+		+						+	R	R	R	S	S	S
3	80/81/KS6				+		+						+	R	R	R	S	S	S
4	80/KS6		+	+		+	+		+	+	+		+	R	R	R	S	S	S
5	80/KS6				+		+			+				R	S	S	S	S	S
6	80/KS6		+	+	+	+			+	+	+			R	R	R	R	R	S
7	80/81			+										R	S	S	S	S	S
8	80/81				+		+							R	R	R	S	S	S
9	80/81				+	+	+							R	R	R	S	R	R
10	80				+		+							R	R	S	S	S	S
11	80													S	S	S	S	S	S
12	80		+	+	+									R	R	R	S	S	S

*See footnotes to Tables 3 and 4.

antibiotic sensitivity patterns. The same applies to the strains of 80/81 and of type 80, although no lysis was observed within each of these; the antibiotic sensitivity of the strains within these two groups was also different.

From these and similar studies it may be concluded that tests for lysogenic properties can add further evidence for the identity or difference of strains yielding similar or identical patterns of lysis with the conventional phages.

Discussion. Staphylococci typing as "80/81" are currently the ones most frequently cultured from staphylococcal infections in many hospitals. Originally found almost exclusively in hospitals, strains of this type are now also very common in the community as well. In spite of the fact that a variety of patterns is often observed with the phages commonly used (namely 80, 81, 52, and 52A), most workers prefer to consider strains lysed by one or more of these phages as identical. However, considering the high frequencies of these strains within hospitals, this failure to distinguish the various lytic patterns within this group may hamper the epidemiological tracing of the origin of hospital outbreaks.

Attempts, therefore, have been made to classify these strains further. Tests employing the conventional phages and also phage KS6, in both the routine test dilutions (RTD) and in 1000 × RTD indicated that strains generally referred to as "80/81" should preferably be classified as "group 80/81" since they could readily be subdivided into a number of distinct patterns, as shown in Table 2. The origin of the variants might be disputed, but lysogenization probably plays an important role. The variants seem to be reasonably stable, the same pattern being generally observed in consecutive strains isolated from any individual subject.

Another method commonly used to classify staphylococci is by their antibiotic resistance patterns or antibiograms. It is probably justifiable for epidemiologic purposes to distinguish penicillin-sensitive from penicillin-resistant strains, even if the phage patterns are identical. However, since nearly all hospital strains typing as "80/81" are penicillin-resistant and the majority are also resistant to tetracycline, this classification is of limited value in this respect. Variants resistant to some of the other antibiotics in common use can occur as mutants following exposure to the antibiotic although such conversion during treatment does not seem to be a common occurrence. Therefore, for epidemiological purposes, strains with distinctly different patterns of antibiotic resistance should probably not be considered as having a common origin.

The present study of the antibiotic susceptibility of strains within the phage types of "group 80/81" revealed a variety of patterns indicating that these strains had different origins. For proper epidemiological classification, it thus seems necessary to take into account the antibiotic susceptibility patterns of strains of staphylococci in addition to their phage types.

Further information can be obtained from the study of the lysogenic properties of strains. Lysis of one strain by the phage carried by another indicates that the two strains are different (Boyd[4], Nahmias *et al.*[8]). Cross culture experiments with a few strains of various 80/81 patterns resulted in a number of lytic reactions indicating lysogenic and lysosensitive variants. Cross culture of apparently identical strains, that is, those with the same phage and antibiotic pattern, did not result in lytic reactions. On the other hand, such reactions did occur when strains with different phage patterns or antibiotic

sensitivity, or both, were similarly tested. This method of comparing strains evidently needs further investigation.

Summary and Conclusions. Staphylococci currently classified as phage type "80/81" are now so frequent that epidemiological deductions from their classification in this manner may be seriously hampered. Attempts were, therefore, made to differentiate strains within this group. By using routine test dilutions (RTD) as well as 1000 × RTD of the conventional phages and phage KS6, a number of distinct patterns could be established. In addition, different antibiotic susceptibility patterns were observed among the strains of each phage pattern. It is, therefore, suggested that for epidemiologic purposes, phage typing of staphylococci be supplemented with tests for antibiotic sensitivity. Test for lysogenicity may further add to the characterization of the strains.

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SUMMARIO IN INTERLINGUA

Studios de Staphylococcos del Phagotypo "80/81"

Staphylococcos classificate currentemente como phagotypo "80/81" ha devenite si frequente que deductiones epidemiologic a base de un classification effectuate in iste maniera pote esser seriemente compromittite. Per consequente le essayo esseva interprendite de subdifferentiar diverse racias intra le gruppo mentionate. Per le uso de dilutiones de essayage routinari e etiam de dilutiones 1000 vices illos de essayage routinari pro le phagos conventional e le phago KS6, un numero de distincte configurationes poteva esser establite. In plus, distincte configurationes de susceptibilitate antibiotic esseva observate in correlation con le distincte configurationes de phagotypia. A base de iste constatationes le proponimento es formulate que—pro objectivos epidemiologic—le phagotypia de staphylococcos deberea esser supplementate per tests del sensibilitate antibiotic. Tests del lysogenicitate es possibilemente de adjuta additional in le characterisation del racias.

A SURVEY OF THE MANAGEMENT OF GASTRIC ULCER IN A GROUP PRACTICE

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The continuing controversy (Bachrach[1]) regarding the proper management of gastric ulcer prompted us to undertake a survey of the gastric ulcer cases in a representative population receiving skilled medical care. The purpose of the inquiry was to determine how gastric ulcer is actually managed by qualified physicians and what might be learned therefrom. To pursue the problem under reasonably satisfactory conditions, we studied the records of a medical group whose members are of sufficiently recent vintage to reflect present-day teaching.

This was necessarily a retrospective study. It is well known that much of the information sought in such a study cannot be obtained from the records, and that much of what *is* obtained is unreliable. Particularly, in the investigation of a disease in which the diagnosis depends almost exclusively on Roentgen-ray examination, scrutiny of the records, without review of the the Roentgen-ray films to verify the diagnosis which has been entered in the files, may lead to grossly erroneous conclusions. Having avoided this pitfall, we believe the findings reported herein provide adequate basis for some pertinent observations regarding current concepts of gastric ulcer in a specialty practice typical for a metropolitan area.

Procedure. The survey included all cases indexed under gastric ulcer in the files of one of the large medical clinics in Southern California. The internists, surgeons and radiologists of this group are all board certified or board eligible; they represent a good cross section of medicine as it is practiced by qualified men.

The essential data from the history, physical examination and progress notes were abstracted on special forms. The films of each upper gastrointestinal Roentgen-ray examination on each patient were reviewed in chronological sequence without knowledge of the fluoroscopic findings or of the interpretation of record. Our interpretations of the radiographic signs were entered on a form prepared for this purpose, and a decision was recorded as to whether the ulcer was or was not definitely benign.

Cases were accepted as proven benign gastric ulcer only if the lesion disappeared completely on Roentgen-ray examination or was identified at operation or necropsy.

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Results. An analysis of the 103 cases indexed under "gastric ulcer" is presented in Table 1. It is at once apparent that any statistics based on the assumption that all cases so indexed were in fact gastric ulcer would be erroneous. For example, suppose it were desired to estimate the frequency of surgery for gastric ulcer in this material. If the number of gastric ulcer patients operated upon were divided by the number of patients carried in the files under that diagnosis, the incidence would be 13%. On the other hand, if we exclude the 14 patients erroneously indexed and the 21 patients whose films, either in the initial reading or on review, did not show a definite gastric ulcer, then even assuming that the 9 patients whose films could not be located had gastric ulcers, the incidence of surgery would be 20%. This figure increases to 27% if we restrict ourselves to the 48 proven cases of gastric ulcer to which our attention in this communication is directed.

Diagnostic Procedures. ROENTGEN-RAY. From Table 2, showing the distribution of the 48 ulcers by sex and by location of the lesion, it is evident that the case material in this series is representative in respect to the ratio of males to females and of body ulcers to antral ulcers.

TABLE 1.—ANALYSIS OF 103 CASES INDEXED UNDER "GASTRIC ULCER"

Category			*Number*
1. Roentgen-ray films could not be found			9
2. Erroneous indexing			14
a. Duodenal ulcer		8	
b. Esophageal ulcer		2	
c. No GI series on record		2	
d. No mention of gastric ulcer in original Roentgen-ray reports		2	
3. Diagnosis of gastric ulcer not proven			11
a. Apparently valid diagnosis but not followed to complete healing, operation or necropsy		8	
b. Questionable ulcer, no further roentgenogram		3	
4. Insufficient evidence of gastric ulcer on film review by present authors			21
a. No, or questionable, gastric ulcer		16	
b. Ulcer in pyloric channel or duodenum		4	
c. Erosive gastritis		1	
5. Diagnosis proven			48
a. Healed at last follow-up		33	
b. Healed, recurred at last follow-up		1	
c. Operation		13	
1) Healed, recurred, healed, surgery for persisting symptoms, no gross ulcer found at operation	2		
2) Healed, recurred, operated, ulcer present	5		
3) Surgery after initial Roentgen-ray examination no test of healing	2		
a. No therapeutic response, fear of malignancy; benign ulcer found	1		
b. Surgery indicated anyway for symptomatic hiatus hernia	1		
4) Failed to heal, operated, ulcer found	4		
d. Necropsy (Died of CVA while on ulcer therapy)		1	
Total			103

TABLE 2.—PERTINENT DATA ON 48 CASES OF PROVEN ULCER

	Male			*Female*			
	Operated	*Not Operated*	*Total*	*Operated*	*Not Operated*	*Total*	*Total*
Antral	2	8	10	3	4	7	17
Body	6	11	17	2	12	14	31
Total	8	19	27	5	16	21	48

RESULTS OF GI X-RAY EXAMS IN G.U. PATIENTS

	BEFORE ULCER DEMONSTRATED	WHILE FOLLOWING ULCER TO HEALING	AFTER HEALING	PATHOLOGICAL FINDINGS
1.		● ◘	◐ ○ ●	ULCER, LESSER CURVE
2.		● ● ○		
3.		● ○	○	
4.	○	● ● ○	○ ○	
5.		● ○		
6.		● ● ● ● ○	○ ● ● ○	NO ULCER SEEN
7.		● ●		ULCER, LESSER CURVE
8.		● ● ○		
9.		●		
10.		● ○		
11.		● ● ○		NO ULCER FOUND
12.		● ○	○ ◐	HEALING ULCER, LESSER CURVE
13.		● ● ● ● ○	● ○ ○	
14.	○	● ○		
15.		● ● ○	○ ○	
16.		● ● ○		
17.		● ● ○	○	
18.		● ○	○ ○ ○	
19.	○	● ● ● ○	○	
20.		● ● ○		
21.		● ● ○	◐ ○ ◐	
22.	○	● ● ○	○	
23.	○ ○	● ○		
24.		● ● ○	○	
25.	○	● ● ●		MULTIPLE ULCERS, LESSER CURVE
26.		● ○	○ ○ ○ ○	
27.		● ● ○		
28.		● ○	○	
29.		● ○	○ ○	
30.	○	● ○	○ ○ ○ ○ ○ ○	
31.		●		ULCER, LESSER CURVE
32.		● ● ● ● ●		ULCER, LESSER CURVE
33.		● ● ○	○ ○	
34.		● ○	○	
35.		● ○		
36.		● ● ● ● ○	○ ●	ULCER, LESSER CURVE
37.		● ○		
38.	○	● ○	○ ○ ○ ○ ○	
39.		●		GASTRITIS
40.	○	● ● ○	○ ●	SUPFCL. ULCER, ANTRUM
41.		● ● ○	○ ○	
42.	○ ○ ○ ○ ○	● ● ● ● ●		ULCER, LESSER CURVE
43.	○	● ● ○	○ ○ ○ ○	
44.		● ● ○	○ ●	ANTRAL ULCER
45.		● ◐ ○	○	
46.		● ○	○	
47.		● ○		
48.		● ● ○	●	

● CRATER DEMONSTRATED
◐ NEW ULCER, DIFF. LOCATION
◐ EQUIVOCAL
○ NO ULCER

Fig. 1

The results of upper gastrointestinal roentgenographic examinations performed in following these patients (Fig. 1) show that more than half of them had at least 4 such examinations. Omitting the 3 patients who came to operation or necropsy after their first and only roentgenogram, almost half had *five* or more examinations. The intervals between examinations were extremely variable. Three patients each had 5 upper gastrointestinal roentgenographic examinations within 4, 5, and 6 months respectively, while in each of 3 patients having 6 such examinations the total elapsed times were 22, 27, and 36 months.

In all 48 patients the interpretation of record was "gastric ulcer" without any qualifying statements to engender a fear of malignancy. The interpretations by the investigative team coincided in all cases, and the results of follow-up confirmed these diagnoses. Thus there was no instance in this limited series in which a carcinoma of the stomach was erroneously diagnosed as a benign ulcer. No great significance is attached to this observation, since the only acceptable way to determine whether gastric cancer is being "undercalled" is not to begin with the known gastric ulcers and see how they were originally diagnosed, but rather to ascertain what the radiological diagnosis had been in proven gastric cancers.

It is impossible to estimate the rate of healing of gastric ulcer in this material because of the extreme variation in the intervals between Roentgen-ray examinations (Table 3). Of 41 ulcers which healed during the observation period, 18 were healed at the time of the first follow-up examination, 19 at the second, one at the third and 3 at the fourth. The possibility was considered that in the cases which were healed at the first follow-up examination the interval between the initial and first follow-up roentgenogram might have been longer than in those in which healing was not complete in the first re-examination; that, in other words, the difference in the number of roentgenograms required before demonstration of complete healing might be the result of more time having been allowed for healing than by such factors as the characteristics of the ulcer or the type of therapy. The data (Table 4) show that in both groups, the first follow-up was done within 6 weeks in 60% of the cases; or the same number of patients in each group was not re-

TABLE 3.—INTERVAL BETWEEN ROENTGEN-RAY EXAMINATIONS DURING TREATMENT OF GASTRIC ULCER

	*Between 1st & 2nd**	*Between 2nd & 3rd*	*Between 3rd & 4th*	*Between 4th & 5th*
Less than 4 weeks	12	10		1
4 to 6 weeks	15	4	2	1
6 to 8 weeks	6	2	1	
2 to 3 months	2	3		1
3 to 4 months	5	3	2	2
4 to 5 months	1			
5 to 6 months	2			
More than 6 months	2	4	1	
Total	45	26	6	5

*The interval between the 1*st* and 2*nd* examinations is that between the initial roentgenogram which disclosed the ulcer and the first follow-up.

roentgenographed until more than 4 weeks after the initial examination.

GASTROSCOPY. This procedure was carried out in 10 patients. In one case the examination was reported as unsatisfactory; in 6 cases no lesion was seen. In 2 cases an ulcer was seen and was pronounced benign, and one case was diagnosed as "probable carcinoma." In none of the cases did the gastroscopic examination alter the radiological diagnosis except in the last mentioned one, where the gastroscopist proved to be in error.

TABLE 4.—INTERVAL BETWEEN INITIAL AND FIRST FOLLOW-UP ROENTGENOGRAM IN 41 PATIENTS WITH GASTRIC ULCERS WHICH HEALED

Interval	*Number healed at first follow-up roentgenogram*	*Number not healed at first follow-up roentgenogram*	*Total*
Less than 4 weeks	5	9	14
4 weeks to 6 weeks	6	6	12
More than 6 weeks	7	8	15
Total	18	23	41

GASTRIC ANALYSIS (TABLE 5). The gastric secretions were tested for HCl in 19 patients. The fasting residuum was found to contain acid in 9 cases; in 5 of these this terminated the test, while among the remaining 4 patients the procedure was continued with collection of basal secretions in 2, histamine stimulation in 1 and alcohol stimulation in the other. The residual secretion was negative for acid in 4 cases; in 2 of these the basal secretion was also negative and no further testing was done; in one, acid was secreted in response to histamine and in one it was not. In 2 cases the residual secretion is not reported but the basal secretion was positive for acid. In one case histamine was apparently given at the outset and resulted in the secretion of acid. The azure-A resin (Diagnex) test was performed in the remaining 3 patients; it was negative in 2 and positive in one. Thus in the 19 cases in which acid was tested for, there were 5 in which no evidence was obtained that the patient could secrete acid. Two of these patients came to surgery and 3 did not. Of the 2 who came to operation, one had been under medical management for 5 years; he was no better after gastric resection than he was before, and it then became evident that the gastric ulcer had not been his primary problem. The other patient was a woman in whom the presence of a large hiatus hernia was considered the primary indication. Therefore, in none of the 5 "achlorhydric" patients were the results of gastric analysis of value in the management of the case.

THERAPY. Hospitalization is not an integral part of the gastric ulcer regimen as administered by this medical group. Only 6 of the 40 patients were hospitalized for medical management of the gastric ulcer, and then only for brief periods to determine whether patients refractory to ambulatory management could be controlled by rest and supervised adherence to the schedule of medications. Thus the maximum period of hospitalization for this purpose was 5 days, since by that time all of these patients had responded, although 4 of the 6 later relapsed and came to operation.

Diet. Rigid ulcer diets of the Sippy

type were infrequently prescribed, as would be anticipated from the fact that the patients were not only ambulatory but, in the majority of cases, at their occupations. The records yielded scanty information regarding the details of the dietary instructions and even less regarding the degree of adherence to the dietary part of the regimen.

or two tablets of antacid "after meals" or "three or four times a day." It was also futile to attempt to ascertain, in regard to anticholinergic medication, whether side effects occurred.

Response to therapy. The progress notes did not, in most cases, convey enough information to permit any conclusions regarding the rate of symptomatic response. Nor can such data be derived indirectly from the rate of disappearance of the ulcer in the roentgenogram because of the wide variation in the interval between the institution of ulcer therapy and the first follow-up Roentgen-ray examination.

TABLE 5.—TYPE OF GASTRIC ANALYSIS AND RESULTS IN TERMS OF PRESENCE OR ABSENCE OF ACID IN 19 CASES OF GASTRIC ULCER

Case No.	*Residual*	*Basal*	*Histamine*	*Alcohol*	*Diagnex*
4	—	—			
6			+		
9		+			
13	—	—	—		
21	+	+			
22	+				
25	+			+	
26	+				
27					+
28					—
31	+	+			
32	+				
33	—		+		
38	+				
39					—
41	+		+		
42	—	—			
45	+				
47	+				

Medication (Table 6). In 20 of the 47 patients receiving antacids and in 18 of the 43 receiving anticholinergics, it was not possible to determine from the records what dosage was prescribed. Among the 27 cases in which the dose of antacid was indicated, it was apparent that in 15 instances the dose was inadequate, consisting of one

TABLE 6.—ESSENTIAL THERAPEUTIC DATA IN 47 CASES

	Number Patients Receiving	*Dose Recorded*	*Dose not Recorded*
Antacids	47	27	20
Anticholinergics	43	25	18

COMMENT. A number of significant observations emerge from this study. First, it spells out why conclusions drawn from the routine survey of past records are fraught with error. The literature on the clinical aspects of gastic ulcer is made up, in the main, of just such surveys, with the result that a number of unjustified conclusions have been promulgated. One of these, namely that a reliable diagnosis of

benign gastric ulcer can be made only under the microscope, is challenged (though not overthrown) by the second significant observation in this study, which suggests that when a competent radiologist interprets the radiological criteria as indicating a benign ulcer, he is usually right. This conforms with the evidence recently submitted from several sources (Browne *et al.*[2], Doll *et al.*[3], Kirsh[4], Schwarz[5]).

The fact that more than one-third of the ulcers in this series were in the antrum illustrates the irrelevance of statements that prepyloric ulcers are more likely to be malignant while ulcers of the corpus are more likely to be benign.

Perhaps the most significant observation in this survey pertains to the amount of Roentgen-ray exposure in the management of gastric ulcer (Fig. 1). The 45 patients in this series who had therapeutic trials had a total of 199 upper gastrointestinal examinations, an average of more than 4 per patient. Almost half of the patients had 5 or more Roentgen-ray examinations; one of them had his 5 examinations at intervals of approximately 3 weeks. This excessive irradiation, which presents a serious economic problem, to say nothing of the potential radiation hazard, stems from a lack of confidence in the initial radiological diagnosis; the clinician seeks reassurance in repeated demonstrations that the ulcer still looks benign while it is being treated or, once healed, when symptoms recur.

The eventual healing of 41 of the 45 ulcers in this series, in spite of the apparent inadequacy of the regimen, appears paradoxical unless it is remembered that this study was limited to those cases in which the diagnosis was proven by microscopic examination or by complete radiographic resolution. But even if we assume failure of healing in the 9 cases with "lost" Roentgen-rays and the 8 patients with apparently valid diagnosis of gastric ulcer who were lost to follow-up, we would still have a healing rate of 41 out of 62, or almost 70%; this suggests that either the regimen was more adequate than the clinical records indicated, or that it does not take much treatment to heal a gastric ulcer. Probably both of these assumptions are correct in regard to many of the ulcers in this series. The matter could be clarified by managing a series of patients as follows: When the radiologist makes a categorical diagnosis of benign ulcer, the patient is not re-examined until after 4 weeks of a regimen of 1) adequate doses of antacids hourly throughout the waking hours for the first 2 weeks, and every 2 hours for the second 2 weeks, 2) a diet of pureed foods for the first week, soft foods the second 2 weeks and bland foods the 4th week, and 3) sedation as indicated in patients under stress. The re-Roentgen-ray examination is to be limited to the area of the ulcer, with the radiologist first given an opportunity to familiarize himself with the previous films.

The application of gastric analysis to the diagnosis of gastric ulcer as encountered in this survey is typical of the situation in the average clinic and clinical laboratory; it is a waste of time and money, and worse than that, it may be misleading to the doctor and detrimental to the welfare of the patient. Operation has often been recommended, and sometimes performed, on the basis of an unconfirmed report of achlorhydria. When the diagnosis is in doubt, all that is required of the laboratory is to determine whether or not the patient secretes acid; the amount of acid is immaterial. To accomplish this, the correct procedure must be selected and meticulously applied. The

azure-A resin test in its present form is unreliable for this purpose. The simplest procedure is as follows: a) a radiopaque nasogastric tube is passed and the fasting gastric content aspirated; b) the presence of acid (pink to Töpfer's reagent) in the fasting residual completes the test; c) if the residuum does not contain acid, the position of the tube in the stomach is verified (and adjusted if necessary) by fluoroscopy, and histamine, 0.01 mg. of the base per kilo, or histalog, 0.5 mg. per kilo is given subcutaneously; samples are drawn every 10 minutes until acid appears, continuing up to 90 minutes before the test is declared negative. A negative result must be confirmed at least once before the diagnosis of achlorhydria is tenable.

Gastroscopy was not helpful in the present series, but it was not done in a sufficient number of cases and particularly in the type of case (radiographically equivocal) where its merits could be evaluated.

Summary. In a retrospective study of the management of gastric ulcer in the practice of a well-qualified medical group it was found that in all of the 48 cases with adequate follow-up, the radiological diagnosis of benign ulcer was confirmed. The methods of gastric analysis followed no consistent pattern and the findings were often ignored. For instance, in the 3 patients in whom tubeless gastric analysis was employed, 2 were reported to be achlorhydric, but the results were not verified by intubation. Gastroscopy was employed too infrequently to be evaluated as a diagnostic aid.

Appraisal of the therapeutic regimen was impossible in most of the cases because of omissions in the records. Detailed entries setting forth the exact instructions given to the patient, including the amounts and intervals of the therapeutic agents, and the symptomatic status of the patient at successive visits, were the exception. The main shortcoming in the regimen was the inadequacy of antacid management. Nevertheless, and without benefit of hospitalization except for a few days in each of a few patients, 41 of the 45 ulcers eventually healed.

Almost half of the patients had 5 or more Roentgen-ray examinations of the upper gastrointestinal tract in connection with their gastric ulcers. Important factors contributing to this excessive number of Roentgen-ray examinations are thought to be 1) a lack of confidence in the reliability of the radiological differential diagnosis between gastric ulcer and cancer, and 2) inadequate attention to the therapeutic regimen, particularly to the importance of antacid therapy, with the result that the healing process was unduly prolonged.

Conclusions. 1) A survey of the management of gastric ulcer in a group practice yielded evidence of too much preoccupation with the radiological status of the ulcer and not enough with the clinical status of the patient. 2) Gastric analysis was of no value in this series because of lack of uniformity of technique.

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SUMMARIO IN INTERLINGUA

Un Analyse del Manipulation de Ulcere Gastric in un Practica de Gruppo

In un studio retrospective del manipulation de ulcere gastric in le practica de un ben qualificate gruppo medical, il esseva trovate que in omne le 48 casos con adequate observationes de controlo catamnestic, le diagnose radiologic de ulcere benigne esseva confirmate. Le methodos de analyse gastric non sequeva un schema uniforme, e frequentemente le constatationes esseva ignorate. Per exemplo, in le 3 patientes in qui analyse gastric sin tubo esseva empleate, le reporto pro 2 esseva que illes esseva achlorhydric, sed iste resultato non esseva verificate per intubation. Gastroscopia esseva usate troppo infrequentemente pro esser evalutate como adjuta diagnostic.

Un evalutation del regime therapeutic esseva impossibile in le majoritate del casos a causa de omissiones in le protocollos. Le presentia de detaliate notationes stipulante exactemente le instructiones recipite per le patiente, incluse le dosage e le horario del agentes therapeutic, e indicante le stato symptomatic del patiente al tempore del successive visitas esseva plus tosto exceptional. Le principal inadequatia in le regime esseva illo del methodos antacidic. In despecto de omne isto—e sin le beneficio de hospitalisation, con le exception de pauc dies in pauc casos individual—41 del 45 casos de ulcere resultava in curation in le curso del tempore.

Quasi un medietate del patientes habeva 5 o plus examines a radios X in le vias gastrointestinal superior in connexion con lor ulceres gastric. Es opinate que importante factores contribuente a iste excessive numero de examines roentgenologic esseva (1) le manco de confidentia in le fidelitate del radiodiagnose differential inter ulcere gastric e cancere e (2) un inadequate grado de attention prestate al regime therapeutic, particularmente al importantia del therapia antacide, con le resultato que le processo curatori esseva prolongate innecessarimente.

Le studio supporta le sequente conclusiones: (1) Un analyse del manipulation de ulcere gastric in un practica de gruppo produceva evidentia de un plus grande preoccupation con le stato radiologic del ulcere e non satis con le stato clinic del patiente. (2) Analyse gastric esseva sin valor in iste serie a causa del manco de uniformitate de technica.

RESULTS OF TREATMENT IN 71 PATIENTS WITH IDIOPATHIC THROMBOCYTOPENIC PURPURA

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The administration of ACTH or corticosteroids (Meyers, Miller, and Bethell[7], Meyers *et al.*[8], Zarafonetis, Steiger, and Cary[10], Dameshek *et al.*[5]) and splenectomy (Coller, Blain and Andrews[2], Orebaugh and Coller[9], Carpenter *et al.*[1]) are accepted as the major effective therapeutic measures in the treatment of idiopathic thrombocytopenic purpura. However, the relative merits and demerits and indications for and against either method of treatment have not been fully resolved. In particular, concern has been expressed regarding 1) the likelihood of increasing postsurgical infection by the preoperative administration of corticosteroids to patients who may require splenectomy (Doan, Bouroncle, and Wiseman[6]) and 2) the possibility of unmasking or causing an exacerbation of systemic lupus erythematosus by splenectomy (Dameshek and Reeves[4], Dameshek[3]).

Since we had not been impressed with hypersusceptibility to either infection or to systemic lupus erythematosus (S.L.E.) as particular consequences of the treatment of idiopathic thrombocytopenic purpura (I.T.P.), we have reviewed our experience at the Simpson Memorial Institute during the 11-year period 1950 to 1961 in the treatment of 71 adolescent and adult patients with I.T.P. Specific attention has been directed to the complications of treatment.

Material and Methods. Criteria for the diagnosis of I.T.P. included thrombocytopenia, increased capillary fragility, prolonged bleeding time, defective clot retraction and a cellular and active marrow with a normal or increased number of megakaryocytes. Serologic tests for syphilis and L.E. cell tests were done on the majority of patients and were negative in each instance. With the exception of 4 patients who had rheumatoid arthritis, all of the patients were clinically free of associated disease to which hypersplenic thrombocytopenia might be ascribed.

Although an initial diagnosis of I.T.P. was made on 92 patients during the period 1950 to 1961, only 71 patients are included in this study. Children under 13 years of age were excluded because of the recognized difference in the natural course of the disorder in children as opposed to adolescents and adults (Carpenter *et al.*[1]) and because we saw too few children to evaluate them as a separate group. Patients with recent viral infections or with questionable drug histories, patients seen in consultation for diagnosis only, patients followed for less than 9 months, and patients lost to follow-up were also excluded. Each of the 71 patients on whom this report is based has been followed either to date or to death (9 months to 11 years).

The series consists of 14 males and 57 females. Distribution by age and sex is shown in Table 1. All had clinically active purpura as well as thrombocytopenia at the time therapy was begun. Four had had splenectomy previously. Treatment in each instance was initiated with either ACTH or a corticosteroid. In general, the daily doses employed were ACTH 100 mg. intramuscularly, cortisone 300 mg., prednisone 60 mg., or methylprednisolone 48 to 60 mg. Treatment was continued for 10 to 21 days. If the response was optimal (complete clinical and hematologic remission), the therapeutic agent was withdrawn over a 4- to 6-day period.

Subsequent relapse was considered an indication for re-treatment and splenectomy. Failure to achieve a complete remission in response to hormone therapy was considered an indication for splenectomy. The chronic administration of a corticosteroid without splenectomy was not favored and was limited to a single elderly woman.

TABLE 1.—AGE AND SEX OF 71 PATIENTS WITH I.T.P. TREATED WITH ACTH OR CORTICOSTEROIDS

Years	*Male*	*Female*
13–20	3	11
21–30	1	17
31–40	5	11
41–50		5
51–60	1	7
61–70	4	4
71–80		2
	14	57

Results. In 31 of the 71 patients, the response to the administration of ACTH or a corticosteroid was excellent (Table 2-A). The platelet values and bleeding times returned to normal and clinical symptoms disappeared. In 10 of the 31 patients, the remissions have been sustained for periods varying from 10 months to 11 years. A single patient with an optimal response to ACTH had immediate splenectomy because of a history of chronic purpura. Twenty of the 31 patients relapsed after cessation of hormone therapy. Two had had splenectomy previously. In 17 of this group a second or third remission was induced successfully, and, while the hemorrhagic diathesis was under control, splenectomy was performed without operative mortality or morbidity. In 15 of the 17 patients, sustained remissions have followed splenectomy. After initial good responses to splenectomy, 2 patients relapsed after 3 weeks and 2 years respectively. Both have required chronic corticosteroid administration for the control of symptoms. One 34-year-old man had a transient optimal response to ACTH but declined further treatment and died at home of cerebrovascular bleeding.

In 40 of the 71 patients (Table 2-B), response to the administration of ACTH or a corticosteroid was suboptimal. Two patients had previously failed to respond to splenectomy. The thera-

TABLE 2.—IDIOPATHIC THROMBOCYTOPENIC PURPURA TREATED WITH ACTH AND CORTICOSTEROIDS

A.	Optimal Remission	*31*
	Sustained	10
	Immediate splenectomy-sustained response	1
	Unsustained	20*
	Re-treatment followed by splenectomy	17
	Sustained remission	15
	Remission followed by relapse	2
	Preoperative death	1
B.	Suboptimal Remission	*40**
	Followed by splenectomy	36
	Sustained remission	30
	No response	4
	Operative deaths	2
	Preoperative death	1
	Chronic purpura	1
	Total number of patients	71
	Number requiring splenectomy	54/71 = 76%
	Number cured by splenectomy	46/54 = 83%

*Two patients in each group had previously had splenectomy.

peutic effect was not materially improved in the 10 patients in whom it was attempted by increasing the daily dose of medication, changing from one preparation to another, or prolonging the treatment period. Thirty-six of the 40 patients had splenectomy while under hormone treatment. Of these, 30 have had sustained remissions following splenectomy, 4 failed to improve, and there were 2 operative deaths. A single patient (Case 1) died of massive intracranial bleeding while being prepared for splenectomy by the administration of prednisone. One older woman with chronic purpura, in whom splenectomy has been withheld, has been maintained in partial remission by the continuous administration of 20 mg. of prednisone daily for 9 months.

Of the 54 patients who were subjected to splenectomy after preparation with ACTH or a corticosteroid, 46 have had complete relief of their thrombocytopenia.

Case Reports. The following 4 cases illustrate respectively the special problems in management imposed by inadequate treatment, incomplete diagnosis, technical complications at surgery, and underlying S.L.E.

CASE 1. N.C., a 20-year-old white construction worker, was admitted on May 4, 1956, with a 5-month history of recurrent but increasingly severe epistaxis, petechiae and purpura. There was no history of recent infection, drug ingestion or toxic exposure. His past health had been excellent.

On physical examination, he was a well-developed and well-nourished young man. Positive findings were limited to evidences of bleeding with moderate epistaxis, petechiae over the skin and visible mucous membranes and bilateral conjunctival and retinal hemorrhages.

The admission hemoglobin was 11.4 gm.%, hematocrit 40%, white blood cell count 16,150 per c.mm. with the following differential: 72% neutrophils, 10% lymphocytes, 5% monocytes, 12% eosinophils, and 1% basophils. No platelets could be found on the stained films. The bleeding time was markedly prolonged. The clotting time was 5 minutes, but there was no clot retraction within 24 hours. The capillary fragility test was strongly positive. The sternal marrow was cellular with active and orderly erythro-and granulopoiesis. Megakaryocytes were abundant but without evidence of platelet budding.

The diagnosis of I.T.P. was made and, on May 5, treatment with prednisone, 10 mg. every 6 hours, was begun. On May 7, the hemoglobin was 6.6 gm.% and the hematocrit 24%. There had been no relief from the hemorrhagic phenomena. On May 9, the hemoglobin was unchanged but occipital headache developed. The prednisone was increased to 15 mg. every 6 hours. At noon a transfusion of 500 ml. of whole blood with 50 mg. of hydrocortisone hemisuccinate was given followed by an intravenous drip containing 200 mg. of hydrocortisone. At 4 p.m. sterterous breathing rapidly followed by coma and areflexia developed. The patient died at 5 p.m.

At necropsy, the major findings were extensive recent left cerebellar hemorrhage, subarachnoid hemorrhage, and bleeding into the myocardium, kidneys and stomach.

COMMENT. In reviewing the possible reasons for failure in the management of this young man, two facts stand out: 1) 40 mg. per day of prednisone is less than our usual dose, 2) the progressive anemia which resulted from continued epistaxis was allowed to go uncorrected. It is regrettable that he did not receive more adequate amounts of corticosteroid and earlier transfusions.

CASE 2. J.B. On June 1, 1958, this 77-year-old white housewife had the onset of epistaxis which was not controlled by packing. Three days later she developed petechiae over the entire body and thrombocytopenia was noted. After failure to respond to the transfusion of 5 units of whole blood and treatment with triamcinolone, she was transferred to the University Hospital on June 10, 1958.

There was a history of night sweats for approximately one month before admission. Her health otherwise had been good.

On physical examination, the temperature was 100°, pulse 112, respirations 24, blood pressure 150/76. She was a pale, thin woman with fresh blood oozing from the right nostril and bilateral subconjunctival hemorrhages. Petechiae were present over the buccal mucosa, face, arms, chest and legs. Several 1- to 2-cm., firm, but freely movable lymph nodes were palpable in the right cervical

area. The remainder of the general physical examination was not remarkable.

The admission hemoglobin was 8.3 gm.%, hematocrit 27.5%, white blood cell count 15,950 per c.mm. with the following differential: 91% neutrophils, 2% lymphocytes, and 6% monocytes. There were no platelets on the stained films. The bleeding time was greatly prolonged. The clotting time was 7 minutes. There was no clot retraction within 19 hours. Prothrombin concentration was 74%. Stained films of sternal marrow aspirate were hypercellular with abundant megakaryocytes but no platelets. Erythro- and granulopoiesis were active and orderly. Histologic sections of the material did not provide additional information. Specifically, it was noted "there is no proliferation of reticuloendothelial elements that would suggest a reticulohistiocytosis."

The diagnosis of I.T.P. was made. Despite methylprednisolone, 48 mg. per day, followed by hydrocortisone, 200 mg. per day intramuscularly, and repeated transfusions of fresh siliconized blood, there was progressive deterioration. On June 17, 1958, an emergency splenectomy was performed. Generalized bleeding continued and death occurred 6 hours postoperatively.

At necropsy the gross findings were those of severe generalized bleeding with massive gastrointestinal hemorrhage. There was slight enlargement of the cervical, mediastinal and mesenteric lymph nodes. On microscopic examination, the enlarged nodes showed Hodgkin's disease.

Sections of the surgically removed spleen, however, revealed no evidence of Hodgkin's disease.

COMMENT. Hypersplenic thrombocytopenia associated with involvement of the spleen in lymphoblastomata is not rare. In this instance, however, it is difficult to invoke Hodgkin's disease as the cause of the thrombocytopenia. Neoplastic changes were not demonstrable in the spleen, and although the Hodgkin's disease was multifocal, the actual volume of tissue involved by the neoplasm was small.

CASE 3. G.B., a 54-year-old white housewife, was admitted to University Hospital on December 25, 1954, with a 3-month history of petechiae, purpura, epistaxis and gingival bleeding which had not responded satisfactorily to either ACTH or cortisone. During the 24 hours preceding admission, she had developed fever, cough and hemoptysis.

On physical examination, the patient was obese. The temperature was 104.2°, pulse 100, respirations 32, blood pressure 130/70. There was active bleeding from the nose and gingivae and fresh petechiae and ecchymoses were present over the trunk and extremities. The remainder of the physical examination was within normal limits.

The admission hemoglobin was 11.5 gm.%, and the hematocrit was 39%. Leukocytes numbered 6,900 per c.mm. with the following differential: 58% neutrophils, 35% lymphocytes, 5% monocytes and 1% eosinophils. The platelets numbered 8,100 per c.mm. The marrow aspirate was cellular. Megakaryocytes were increased but platelets were rare. Chest roentgenogram was negative. Blood cultures yielded no growth and sputum cultures no pathogens.

On penicillin, her fever declined over a 5-day period without change in the bleeding tendency. Subsequently ACTH, 100 mg. per day, was given for 14 days, with reduction in the spontaneous bleeding but a maximum rise in platelets to only 60,200 per c.mm. On January 19, 1955, splenectomy was performed. Technical difficulty encountered in maintaining hemostasis of the splenic vein and artery resulted in an estimated blood loss of 2,000 ml. and damage to the tail of the pancreas. A drain was placed, penicillin and streptomycin given, and the ACTH tapered and discontinued. The platelet count ranged from 170,000 to 300,000 per c.mm. She did well until February 6, 1955, when a left subdiaphragmatic abscess became manifest. Following incision and drainage of the abscess, her course was uneventful and she was discharged on March 6, 1955, continuing to drain through a catheter in the left subphrenic space.

She was re-admitted on March 22, 1955. Four days earlier she had had the onset of epigastric pain with nausea and vomiting followed by tarry stools. The catheter had extruded itself one week before the onset of pain. There had been no recurrence of epistaxis or bruising.

On physical examination, she was in severe discomfort with epigastric pain. The temperature was 100.2°, pulse 110, respirations 20, and the blood pressure 146/90. The skin and visible mucous membranes were clear save for a crust at the previous drainage site. The left diaphragm was fixed and elevated. There was diffuse upper abdominal tenderness with rebound tenderness.

The hemoglobin was 14 gm.%, and the

white blood cell count was 20,900 per c.mm. with the following differential: 60% segmented neutrophils, 15% band neutrophils, 1% metamyelocytes, 23% lymphocytes, and 1% monocytes. There were moderate toxic changes in the neutrophils. The platelet count was 175,000 per c.mm. Chest roentgenograms revealed extensive left pleuritis, but Roentgen study of the upper gastrointestinal tract was negative.

Her abdominal pain became more diffuse and more severe. On March 24, 1955, a celiotomy was performed. There was mesenteric thrombosis with massive infarction of the jejunum and ileum. A jejuno-iliostomy was established. The operation was not attended by unusual bleeding. The postoperative course was characterized by rising temperature, unstable blood pressure and stupor. She died on March 30, 1955, 6 days postoperatively.

At necropsy, there was a left subphrenic abscess, generalized acute fibrinopurulent peritonitis and mesenteric thrombosis with infarction of all the remaining small bowel.

COMMENT. This patient appeared to be a reasonably good operative risk save for moderate obesity. The difficulties in establishing hemostasis at the time of splenectomy was primarily the consequence of technical difficulties rather than of a coagulation defect. The combination of local hemorrhage and damage to the pancreas afforded a fertile bed for the development of the abscess which was the basis for her subsequent fatal complications. In light of the magnitude of the surgical complications, it does not seem reasonable to incriminate the hormones she received preoperatively in her death.

CASE 4. L.R. This white housewife was first seen at the University Hospital in 1946 at the age of 24 with a 4-year history of rheumatoid arthritis. Routine laboratory studies of the blood, including the Kahn test, and urine were normal. A conservative program of salicylates and physical therapy was recommended.

She returned in May, 1952, complaining of a bleeding tendency. In the interim since her 1946 visit, the activity of her arthritis had fluctuated but not increased. Hypermenorrhea developed in 1951 and was not corrected by uterine dilatation and curettage. Petechiae and epistaxis were first noted early in 1952. The diagnosis of I.T.P. was made and she was treated for the first time with cortisone with improvement. However, relapse followed promptly upon withdrawal of the cortisone. After re-treatment with cortisone, the spleen and 14 accessory spleens were removed with correction of the thrombocytopenia. On microscopic examination of the splenic tissue, there was no evidence of S.L.E.

She did well, save for mild arthritis, until 1955 when petechiae, hypermenorrhea and thrombocytopenia recurred and constant corticosteroid therapy became necessary for the control of bleeding. In 1956, the removal of two additional accessory spleens did not affect the purpura.

In the summer of 1956, an irregular erythematous rash was transiently present over the nose and malar regions. L.E. cell tests performed by two techniques were negative.

In 1958, she required gastric resection for the control of a bleeding gastric ulcer. Attempts to reduce the corticosteroid dose were unacceptable to the patient because of severe flares in the arthritic manifestations. Latex and sheep cell tests for rheumatoid factor and L.E. cell tests were negative but the p-toluene sulphonic acid test was repeatedly positive.

In 1959, the L.E. cell test became positive. Convulsions, pericarditis, pleuritis and chronic ulcerations over the fingers, palms, and buttocks developed.

During 1960, the thrombocytopenia, polyserositis and arthritis were relatively quiescent but she became completely incapacitated by intractable pain from the persistent ulcers. Microscopic examination of biopsy material from the ulcers revealed active chronic productive ulceration in the subcutaneum but no evidence of vasculitis. Tests for cold agglutinins, macroglobulins and cryoglobulins were negative. The Kahn test remained negative.

COMMENT. This patient's S.L.E. evolved slowly over a long period. If the arthritis, which had its onset in 1942, is accepted retrospectively as her first manifestation of S.L.E., she has had S.L.E. for 18 years. Since 4 years elapsed after splenectomy before there was significant increase in the activity of the S.L.E., we do not feel that splenectomy accelerated her deterioration. However, it does seem rea-

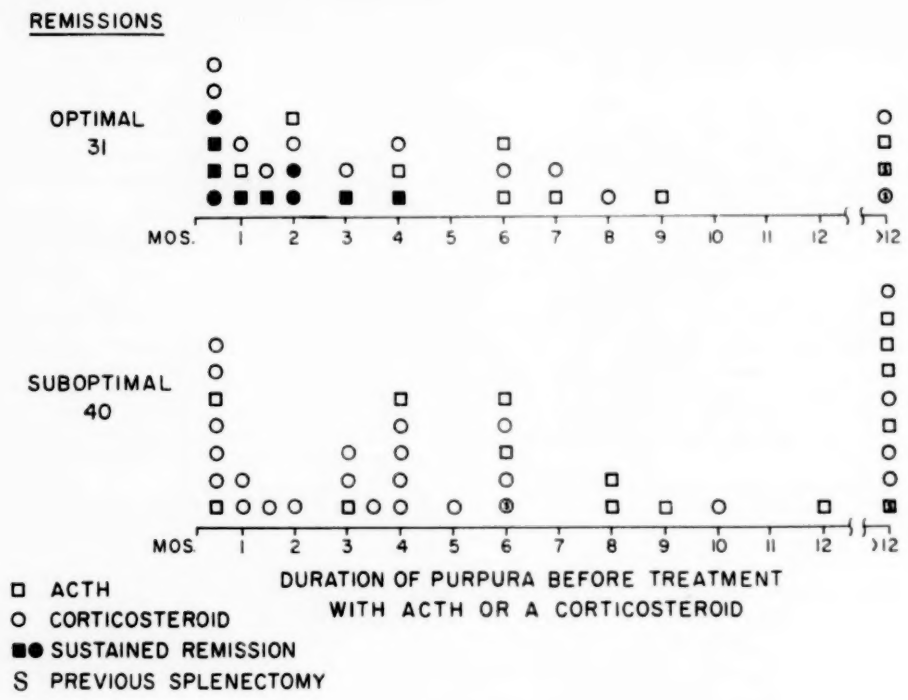
REMISSIONS
OPTIMAL
31
MOS. 1 2 3 4 5 6 7 8 9 10 11 12 >12
SUBOPTIMAL
40
MOS. 1 2 3 4 5 6 7 8 9 10 11 12 >12
DURATION OF PURPURA BEFORE TREATMENT
WITH ACTH OR A CORTICOSTEROID
□ ACTH
○ CORTICOSTEROID
■● SUSTAINED REMISSION
S PREVIOUS SPLENECTOMY

Fig. 1

sonable to implicate the protracted administration of corticosteroids in the pathogenesis of the bleeding gastric ulcer that necessitated resection. In this series, 2 additional patients requiring long-term treatment with hormones developed bleeding ulcers. In contrast, no instance of peptic ulceration was recognized among patients who received the hormones for short periods (that is, 2 to 4 weeks).

Discussion. The 10 patients who have had sustained remissions from hormone therapy alone shared two features in common: their purpura was of short duration before the initiation of hormone therapy and their responses to hormone therapy were optimal (Fig. 1). No sustained remissions from hormone therapy alone have occurred in patients with purpura of more than 4 months duration before the initiation of treatment. No significant therapeutic advantage among the various hormones has been evident. Age and sex have been without apparent influence on the duration of hormone-induced remissions (Table 3).

TABLE 3.—DISTRIBUTION BY AGE AND SEX OF PATIENTS WITH SUSTAINED REMISSIONS FOLLOWING ACTH OR CORTICOSTEROID ADMINISTRATION

Years	*Male*	*Female*
13–20	1	
21–30		2
31–40		1
41–50	1	1
51–60		2
Over 61	1	1
	3	7

Eleven patients in the series have died (Table 4). The two preoperative deaths have been commented upon above. The two operative deaths bear special scrutiny. One (Case 2) occurred 6 hours after splenectomy and was the direct result of failure to correct thrombocytopenia and control bleeding. Necropsy proved the initial diagnosis of idiopathic thrombocytopenia to be incomplete; the patient had associated undetected Hodgkin's disease. The second patient (Case 3) died two months after splenectomy of infection which was directly ascribable to technical difficulties at the time of operation. Her thrombocytopenia had been corrected by splenectomy. Two patients died of recurrent thrombocytopenia with bleeding 2 years and 10 years respectively after splenectomy. In neither was there any evidence of S.L.E. at necropsy.

TABLE 4.—DEATHS AMONG 71 PATIENTS WITH I.T.P.

Preoperative		2
Early postoperative		
Immediate (6 hours)		1
Delayed (2 months)		1
Late postoperative (>2 years)		
Purpura		2
Unrelated		5
Cardiovascular disease	4	
Gastrointestinal disease	1	
Total number of deaths		11
Number related to purpura		6

Five patients died 2 to 9 years after the successful treatment of I.T.P. from entirely unrelated disorders.

Significant nonfatal infection in the immediate postoperative period developed in only 3 patients and in each instance was readily controlled. One patient developed a urinary tract infection, one a gluteal abscess at the site of injections, and another an abscess in a hematoma in the splenectomy incision.

Systemic lupus erythematosus has been encountered in only 2 patients in this series. In one whose thrombocytopenia had been relieved by splenectomy, the diagnosis of S.L.E. was made solely on the basis of the histopathological findings in the spleen. No cutaneous, musculoskeletal, serosal, renal, biochemical or serologic findings of S.L.E. have developed over a 10-month period of observation. In the other patient (Case 4), the gradual evolution and multiplication of frank manifestations of S.L.E. were not unexpected despite the absence of diagnostic changes in the spleen. Her thrombocytopenia was superimposed on long-standing rheumatoid arthritis and was not relieved by splenectomy. Among the other 3 patients with rheumatoid arthritis, splenectomy has corrected the thrombocytopenia and repeated studies for specific evidences of S.L.E. have been negative, albeit one young woman has developed scleroderma.

Summary and Conclusions. The response to treatment of 71 adolescent and adult patients with idiopathic thrombocytopenic purpura (I.T.P.) followed between 1950 and 1961 has been reviewed.

1. In 10 patients (14%), treatment with ACTH or a corticosteroid has been followed by sustained remission. Criteria for identifying patients who may have prolonged remissions following hormone therapy alone are suggested.

2. In preparation for splenectomy, ACTH and the corticosteroids have been valuable agents in establishing hemostatic control.

3. Splenectomy has been followed by prolonged remissions in 46 of 54 (83%) patients.

4. The preoperative administration of ACTH or corticosteroids to patients who required splenectomy was not attended by unduly severe or frequent postoperative infection. The single fatal infection in this series was primarily the consequence of technical difficulties encountered at surgery.

5. Splenectomy has not enhanced the subsequent evolution of systemic lupus erythematosus in patients firmly diagnosed as having I.T.P.

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SUMMARIO IN INTERLINGUA

Resultatos Therapeutic in Septanta-Un Patientes con Idiopathic Purpura Thrombocytopenic

Es presentate un revista del responsas therapeutic constatate in 71 adolescente e adulte patientes con idiopathic purpura thrombocytopenic qui esseva observate inter 1950 e 1961.

1. In 10 patientes (14%), le tractamento con ACTH o un corticosteroid esseva sequite per un sustenite remission. Es suggerite criterios pro le identification de patientes in qui prolongate remissiones es possibilemente effectuabile per un therapia hormonal sol.

2. In preparation pro splenectomia, ACTH e le corticosteroides se ha provate de alte valor como agentes estabiliente le requirite dominio hemostatic.

3. Splenectomia esseva sequite per prolongate remissiones in 46 ex 54 casos (83%).

4. Le administration pre-operatori de ACTH o corticosteroides a patientes requirente splenectomia non esseva associate con infectiones post-operatori de severitate o frequentia excessive. Le solitari infection mortal in iste serie esseva primarimente le consequentia de difficultates technic incontrate durante le operation.

5. Splenectomia ha non promovite le subsequente evolution de systemic lupus erythematose in patientes con definite diagnoses de idiopathic purpura thrombocytopenic.

THE ROLE OF A HEMOLYTIC SYSTEM IN HUMAN TISSUES IN SOME HEMOLYTIC STATES*

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The mechanism through which the erythrocyte meets its destruction in normal conditions remains controversial. In pathologic conditions, many mechanisms have been found significant. Chemicals, drugs, and parasites may destroy the red cell directly within the circulation. Autoantibodies may also exercise a direct lytic activity, or they may agglutinate or damage red cells and thus make them more susceptible to intraorgan mechanisms such as phagocytosis. There are, however, instances where no such mechanisms are to be found and the pathogenesis of the increased red cell destruction remains obscure.

Attention has been directed to tissue factors to explain the final steps in the physiologic destruction of erythrocytes and the otherwise unexplained hemolysis of many pathologic conditions. Weil[26] found weak red cell lysins in normal tissues and in tumors free of necrosis. He found more active and more easily soluble lysins in necrotic tumors. He postulated the possible importance of tissue lysins in the normal destruction of red cells, in "idiopathic" hemolytic anemia, and in the anemia of patients with cancer. Since then, it has been found that tissue lysins, obtained by extracting tissues and plasma with organ solvents, are a heterogenous group of substances. One such hemolytic agent, which may be obtained by extracting tissues and blood with alcohol, petroleum ether and chloroform, is a mixture of phospholipids and soaps of fatty acids (Brinkman[6]) which is readily soluble in cold ether. Another lytic substance, relatively insoluble in ether, may be obtained by extracting plasma and tissues with alcohol; it is now identified as lyso-lecithin (Bergenhem and Fahreus[2]).

Other potent tissue hemolytic agents have been isolated in recent years, such

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as a nonsaturated, monocarboxylic fatty acid with a chain length of 18 C (Laser[17]) and a nonsaturated fatty acid (cis-octadeic-11-enoic-acid), most active when mixed with oleic acid (Morton and Todd[20]). Similar chemicals have been obtained from brain, liver, spleen and normal human serum (Laser and Freeman[18]). These chemical studies are well correlated with many observations on the relationship between hyperlipemia and increased hemolysis, and on the effect of various lipid fractions on normal red cells *in vitro* and *in vivo* (Bodansky[5], Freeman and Johnson[12], Freeman, Lahey and Johnson[13], Longini and Johnson[19], Stocks[23], Swank and Rath[24]). From a clinical viewpoint, there is a well established relationship between dietary fat intake and degree of hemolysis in patients with chronic idiopathic hemolytic anemia (Evans[11], Johnson, Freeman and Longini[16]).

The mechanism of action of tissue lysins on red cells is not entirely clear, possibly because of the variability of their chemical nature (Ponder[21]). Under normal conditions, the activity of lysins may be of little or no importance since they are not free as such in tissues. Their activity is further limited by the presence of specific inhibitors in tissues and plasma. The inhibitors also are of different chemical nature. They have been identified with proteins, lipids, lipoprotein complexes, cholesterol and other substances, all firmly bound to tissues. The relationship of lysins to inhibitors is obscure. Tissues probably contain lysin-inhibitor complexes. The lytic component must be split from the inhibitor to become active. *In vitro,* this is done by the crude but effective method of the extraction with various fat solvents; *in vivo,* possibly through enzymatic activities.

Once the tissue lysin is freed, it may affect the erythrocyte in many ways. The incubation of normal red cells with the splenic pulp from patients with hemolytic anemia causes increased osmotic and mechanical fragility and, in some cases, may lead to the coating of the erythrocytes with a substance reacting with antihuman globulin serum (Wagley *et al.*[25]). On the other hand, the intrinsically abnormal red cells show increased spheroidicity and osmotic fragility when trapped in normal spleens (Weisman *et al.*[27]). These facts essentially show that tissue factors, presumably lysins, may damage the red cell, whether normal or abnormal. It is also conceivable that, if in high concentrations, they may directly destroy erythrocytes which have been made spherocytic by hypoxia in the spleen or in other sluggish areas of the circulation.

It is the purpose of this article to discuss some findings collected over the past 6 years pertaining to a hemolytic system of tissues in normal individuals and in a group of patients with hemolytic anemia where *immunologic mechanisms were not in evidence.*

Materials and Methods. Four "normal" spleens were obtained from individuals either because of traumatic rupture (2 cases) or because of intercurrent procedures (gastrectomy, 2 cases). Pathologic spleens were obtained from 20 patients, most of them as surgical specimens. Various other organs were obtained at necropsy from 5 individuals without evidence of hemolytic anemia. Red cell stroma was obtained by the method of Ballantine from blood of normal subjects. The presence of active clinical hemolysis was established with the usual criteria including blood counts, reticulocyte count, red cell survival time by $NaCr_{51}$ method, determination of fecal and urinary urobilinogen, and others. Immune mechanisms were sought by the study of red cell agglutinins at 37° C. and 4° C., Coombs test (direct and indirect) and antibody elution techniques (Dacie[7]). The cases selected were those where no evidence could be found of immune mechanisms.

(a) COLLECTION AND STORAGE OF TISSUES.

Surgical specimens were promptly cut in small pieces, rinsed in cold 0.9% NaCl solution until the washing fluid was no longer stained with blood, and frozen at −20° C. until used. In some cases, the entire spleen was quickly rinsed with cold saline solution and frozen in liquid nitrogen. Necropsy specimens were obtained within 2 hours of death, cut, rinsed with cold saline and frozen at −20° C. until used.

(b) EXTRACTION OF TISSUE LIPIDS. After thawing at 4° C., tissue pieces were rinsed again 4 to 6 times with cold saline and homogenized in a Potter homogenizer. The homogenate was weighted and centrifuged at 3,000 rpm for 10 minutes at 4° C. The sediment was extracted with benzene (5 ml. of solvent per 1 gm. of wet tissue) (Fig. 1). After centrifugation at 3,000 rpm for 15 minutes at 4° C., the supernatant benzene was decanted and the solid residue treated with 5 volumes of a 4:1 mixture of chloroform-methanol. The liquid part was collected by filtration through glass wool. Samples of the benzene extract (*containing most free lipids*) and of the chloroform-methanol extract (*containing most protein-bound lipids as well as phospholipids*) were set aside. They were dried under 26" vacuum at 25° C.

Further separation of tissue lipids was obtained by fractionation of a mixture of both extracts by chromatography on silicic acid column* (Fig. 2). Glass columns were prepared from 1.8 mm. glass tubing drawn to a delivery tip at one end and a plug of glass wool introduced at this end to support the absorbent. Five grams of silicic acid (Mallinckrodt, activated at 120° C. for 24 hours) and 2½ gm. of Celite analytical filter aid were suspended in chloroform and transferred to the glass column. The absorbent was packed using pressure from a tank of compressed nitrogen, without allowing the solvent to fall below the surface of the column. Approximately 100 mg. of the lipid mixture were dissolved in 20 ml. of chloroform and poured onto the column. The chloroform eluate (*eluate 1*) was collected by gravity in a Kjeldahl flask and contained most neutral fat, fatty acids, cholesterol and esters. One hundred milliliters of methanol were then poured onto the column. This eluate, also collected in Kjeldahl flask, contained most phospholipids (*eluate 2*). Both eluates were dried at room temperature under 25" vacuum. The separation of neutral fat, cholesterol and fatty acids was carried out with a minor modification of the second step of the method of Bergström[3]. The dry chloroform eluate was dissolved in 20 ml. of petroleum ether in a separatory funnel. Twenty milliliters of a solution containing equal volumes

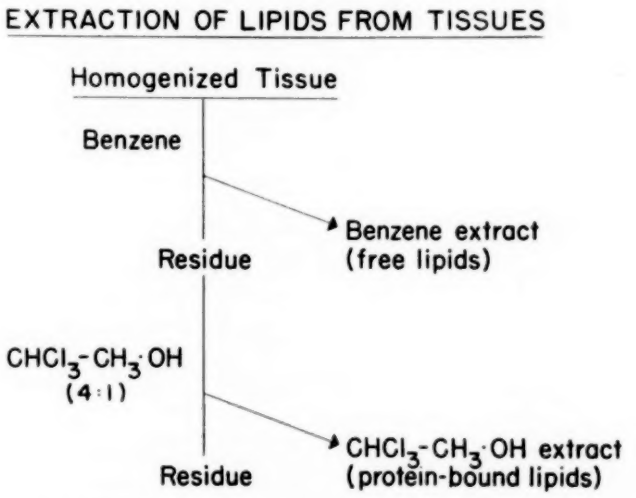

Fig. 1.—Preliminary extraction of tissue lipids (see text).

*Earlier attempts with paper chromatophile (Hawk[14]) were unsatisfactory.

of 50% ethanol and 50 ml. of 0.1 N NaOH, with a few drops of 1% thymol blue were added. After shaking, the alcohol layer was transferred to a second funnel containing 20 ml. of petroleum ether. The mixture was again shaken and the alcohol layer (containing fatty acids) was transferred to a new separatory funnel, acidified with 30% HCl to a red color and extracted with 20 ml. of petroleum ether. After separation of the alcohol layer, the petroleum layer contained neutral fat and cholesterol. The chromatographic separation finally yielded 3 fractions, free fatty acids, neutral fat and cholesterol, and phospholipids. The fractions were dried at room temperature under 26" vacuum, redissolved in a small volume of petroleum ether and stored in the deep freeze at −20° C. under an atmosphere of nitrogen.

(c) PREPARATION OF RED CELL SUSPENSIONS. Human hemoglobin was prepared as a 1% solution in 0.9% NaCl by the method of Drabkin[10] and dried under vacuum. Normal human red cells of group 0 Rh° were washed with saline solution at room temperature and resuspended in saline to contain 3 gm.% hemoglobin.

(d) PREPARATION OF NORMAL HUMAN PLATELETS AND LEUKOCYTES. Human platelets were prepared in saline suspension (Stefanini and Silverberg[22]), containing 300,000 to 500,000 platelets per c.mm. Leukocytes were prepared as described by Dausset, Nenna and Breey[8], washed twice with cold saline and resuspended in saline to give a final suspension of 4,000 to 5,000 cells per c.mm.

(e) ASSAY OF THE HEMOLYTIC AND INHIBITORY ACTIVITY OF TISSUE FRACTIONS. Aliquots of each lipid fraction were resuspended in 1 ml. of isotonic barbital buffer at pH 7.5. A control test tube contained 1 ml. of barbital buffer. Red cell suspension (0.08 ml.) was then added to all tubes in a 37° C. water bath. The time needed for complete hemolysis was recorded, the tubes being agitated every few minutes. The hemolytic activity of each fraction was expressed in units, *one unit of hemolysis being defined as that amount of a lipid fraction inducing complete hemolysis in 30 minutes*. One extract was set aside as standard and dilution curves were prepared. It then became possible to quantitate roughly the amount of hemolytic activity in each extract tested as compared with the standard extract.

The hemolysis inhibitory activity was determined with the same system. Equal vol-

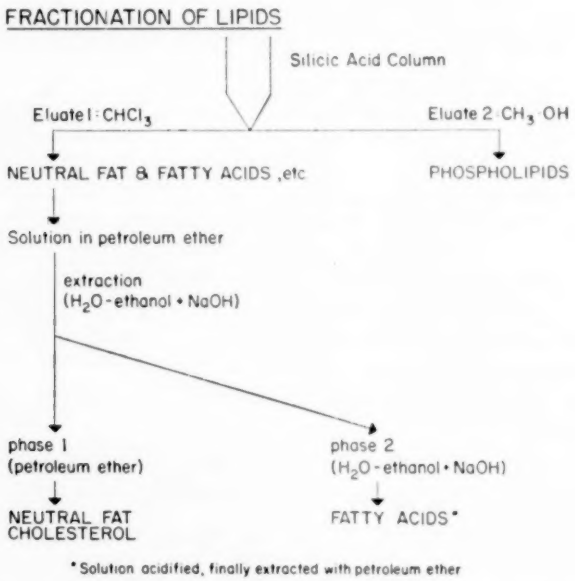

Fig. 2.—Chromatographic fractionation of tissue lipids (see text).

ume of the lipid fraction to be assayed and of a standard suspension of 2 units hemolytic activity were mixed. *One inhibitory unit was defined as that amount of a lipid fraction able to inhibit the activity of one hemolytic unit.*

The lytic activity against white cells and platelets was tested by similar techniques. From 1 to 5 units of hemolytic fraction in 1 ml. of barbital buffer at pH 7.5 were added to 0.1 ml. of suspension of platelets or of leukocytes in test tubes in a 37° water bath. One-tenth milliliter of platelet or leukocyte suspension in 1 ml. of barbital buffer pH 7.5 served as controls. Platelet counts were made using a phase contrast microscope at the beginning and after 30 minutes of incubation as 37° C. The results were checked against Wright stained smears. White blood counts were made at the beginning and after 30 minutes of incubation. Stained smears were used to confirm the results, when needed.

(f) OSMOTIC FRAGILITY AND PRODUCTION OF SPHEROCYTOSIS. Normal human red cells (washed 5 times with saline) were incubated with a subhemolytic amount of the fraction tested for 30 minutes in a water bath at 37° C. The same amount of red cells, incubated 30 minutes without the hemolytic fraction, represented the control. At the end of the incubation period, the hemoglobin in the supernatant was determined by the method of Bing and Barker[4] to rule out significant hemolysis. An aliquot of red cells was centrifuged, cells smeared and stained with Wright's stain. Spherocytes were then counted as per cent of normal cells. The same procedure was followed to show the effect of the inhibitory fractions. Red cell suspension in barbital buffer, incubated and centrifuged in the same manner served as control. Another aliquot of incubated red cells was then washed three times with saline and the osmotic fragility determined by a standard technique (Wintrobe[28]). The same method was used to investigate the effect of inhibitory fractions.

(g) CLASMATIC SEPARATION OF THE UNSATURATED FATTY ACIDS. Two condensers, approximately 8 inches in length, were placed in vertical series over a small spherical distilling flask of 50 ml. capacity. A third longer condenser was connected horizontally with the system through a glass tubing part carrying a thermometer. The horizontal condenser was connected with an Erlenmeyer flask and this to a vacuum pump. The fatty acids extracted from 500 gm. of normal splenic tissue as described were used for the distillation. The fatty acids were desiccated under vacuum and esterified by solution in absolute methyl alcohol (reflux for 3 hours in a water bath at 70° C. with absolute methyl alcohol, using 4% sulfuric acid as catalyst [Deuel[9]]). The methyl esters did not lose their hemolytic activity during manipulation.

(h) MISCELLANEOUS TECHNIQUES. Using the methods discussed by Hawk, Oser and Summerson[15], determinations were made of sodium, potassium and glucose in red cells and fluids. Direct readings were made of the pH values. The amount of phosphorus in various lipid fractions was determined.

Results. (a) NATURE OF THE HEMOLYTIC AND INHIBITORY ACTIVITY OF TISSUE LIPID FRACTIONS. (FIG. 3). The hemolytic activity was recovered in the benzene extract of all tissues tested, and was apparently linked to the free fatty acid fraction. Treatment of the free fatty acid fraction with potassium permanganate (the oxidized material was re-extracted with petroleum ether) resulted in loss of activity, suggesting that this hemolytic activity may be related to the unsaturated fatty acids. The clasmatic separation studies are summarized in Table 1. The free fatty acid fraction of splenic tissue with hemolytic activity contained palmitic oleic, linoleic and arachidonic acids. Arachidonic acid methyl ester exhibited strong hemolytic activity; linoleic and oleic acid methyl esters only slight activity. Thus, arachidonic acid represented probably the main hemolytic agent. Inhibitory activity was present in the chloroform-methanol portion of the extract of tissues, in the cholesterol and neutral fat and in the free phospholipid fractions. Human serum also exhibited powerful inhibitory activity.

(b) COMPARATIVE HEMOLYTIC AND ANTIHEMOLYTIC ACTIVITY OF NORMAL TISSUES (TABLE 2). The organs and tissues studied included brain, kidney, liver, lung, spleen and red-cell stroma. The benzene extract was used as the source of the hemolytic activity and

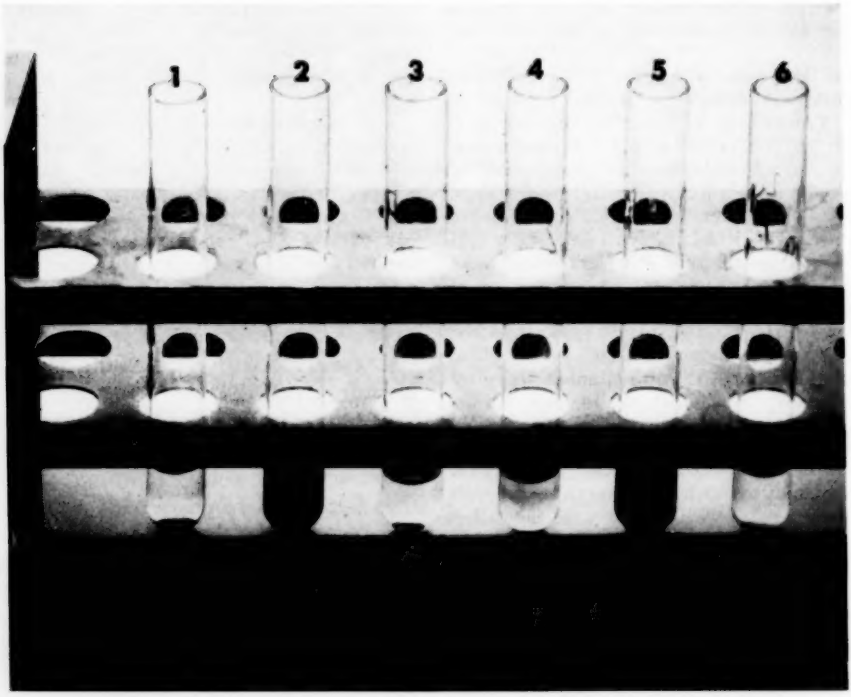

Fig. 3.—A correlation between chemical nature, hemolytic and hemolysis inhibitory activity of tissue lipid fractions*

*From left to right the test tubes contain a mixture of red cell suspensions and the following reagents: (1) barbital buffer (no hemolysis); (2) distilled water (complete hemolysis (3) neutral fats (no hemolysis); (4) free phospholipids (no hemolysis); (5) fatty acids (complete hemolysis); (6) a mixture of fatty acids and phospholipids (no hemolysis). For technique used, see text.

TABLE 1.—THE CLASMATIC SEPARATION OF METHYL ESTERS OF FATTY ACIDS FROM NORMAL SPLENIC TISSUE AND THEIR HEMOLYTIC ACTIVITY

*Boiling Point, °C.**	*Hemolytic Activity*	*Tentative Identification*
136	−	Methyl palmitate
149.5	+	Methyl linoleate
152.5	±	Methyl oleate
160	++	Methyl arachidonate

*Boiling points were checked using pure samples of each fatty acid.

the chloroform-methanol extract as the source of the antihemolytic activity. The hemolytic activity was highest in brain tissue and lowest in red cell stroma. The inhibitory activity was highest in brain and splenic tissue.

(c) THE HEMOLYTIC AND INHIBITORY ACTIVITY OF NORMAL AND PATHOLOGIC SPLEENS (TABLE 3). Splenic tissues from most of 20 patients with various hematological diseases showed hemolytic activity higher than normal, this being especially significant when active hemolysis was present. The tissue in-

TABLE 2.—HEMOLYTIC AND INHIBITORY ACTIVITY OF VARIOUS NORMAL TISSUES (AVERAGE VALUES OF 5 DETERMINATIONS)

	Hemolytic Activity	*Inhibitory Activity*
	units/gm. of wet tissue	
Brain	17.8	125.4
Kidney	8.5	17.3
Lung	6.5	14.6
Liver	5.5	16.5
Spleen	4.8	113.0
Red cell stroma	0.5	8.3

TABLE 3.—HEMOLYTIC AND INHIBITORY ACTIVITY OF SPLENIC EXTRACTS

Case No.	*Hemolytic Activity (units/gm. wet tissue)*	*Inhibitory Activity*	*Surgical Specimen*	*Clinical Hemolysis*	*Steroid Treatment*
(A) *Normal spleens*					
1.	6	135	+		
2.	3	115	+		
3.	6	100	+		
4.	4	90	+		
5.	5	120	+		
Average	4.8	113			
(B) *Pathologic spleens*					
1. Mediterranean anemia	24	5	+	++	No
2. " "	33	1	+	++	No
3. Congenital spherocytosis	53	3	+	++	No
4. " "	25	6	+	++	No
5. Aplastic anemia	21	26	+	++	Yes
6. " "	18	32	+	++	Yes
7. Idiopathic leukopenia	17	29	+	0	No
8. " "	29	37	+	0	Yes
9. Idiopathic thrombocytopenic purpura	8	50	+	0	Yes
10. " "	15	30	+	0	Yes
11. " "	3	21	+	0	Yes
12. Subacute myelogenous leukemia with hypersplenism	17	13	+	+	Yes
13. Hodgkin's disease	47	25	+	++	Yes
14. " "	55	34	+	++	Yes
15. " "	25	53		0	Yes
16. " "	28	46		0	Yes
17. Lymphosarcoma	16	7	+	++	Yes
18. Reticulum cell sarcoma	41	3		++	No
19. " " "	23	54	+	++	Yes
20. " " "	47	27	+	++	Yes

hibitory activity was reduced in all pathologic cases, the lowest values being found in cases who had received no treatment with steroids and where hemolysis was very severe.

(d) THE MECHANISM OF RED CELL DAMAGE BY THE HEMOLYTIC FRACTION. The incubation with subhemolytic amounts of hemolytic fractions increased the initial osmotic fragility of normal human red cells, while the incubation with inhibitory fractions increased their osmotic resistance (Table 4). Many spherocytic cells were visible on stained smears after 30 minutes of incubation of normal erythrocytes with subhemolytic amounts of tissue fractions (Fig. 4). To see whether the production of spherocytes preceded hemolysis, suspensions of normal human erythrocytes were incubated with increasing amounts of hemolytic fraction

TABLE 4.—THE EFFECT OF HEMOLYTIC AND INHIBITORY SPLENIC FRACTIONS ON THE OSMOTIC FRAGILITY OF NORMAL RED CELLS (SEE TEXT)

Incubating Agent	*Saline*	*Hemolytic Fraction*	*Inhibitory Fraction*
		*Osmotic Fragility**	
Initial	0.44	0.72	0.36
Final	0.36	0.32	0.32

*% NaCl solution

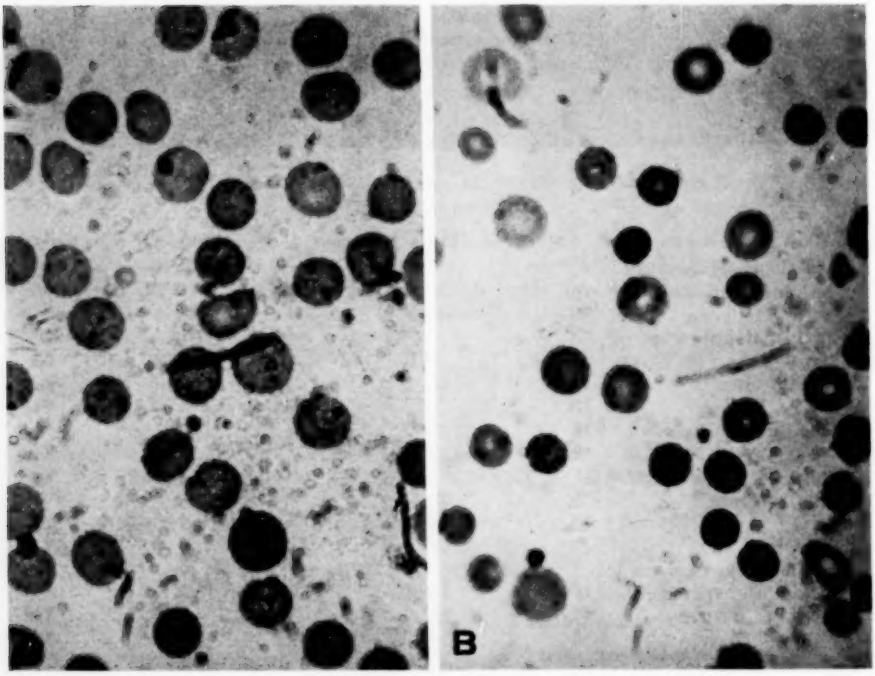

Fig. 4.—The morphology of normal washed human red cells after incubation with (*a*) barbital buffer; (*b*) hemolytic fraction in subhemolytic amounts. Note the marked spherocytosis in (*b*).

from normal human spleens. After one-half hour of incubation, the number of normal and of spherocytic red cells were counted in each sample. Two units of hemolytic fraction or less caused no change in the number of erythrocytes, but a progressive rise in the number of spherocytes. With 3 units of hemolytic fraction, spherocytes dropped as the indicating greater susceptibilitiy of abnormal red cells to the splenic lysins. Four milligrams of glucose fully inhibited the activity of one hemolytic unit. The addition of ascorbic acid, on the other hand, at concentrations up to 2 mg. per ml. did not affect the activity of the hemolytic system. The mechanism of the effect of the hemolytic frac-

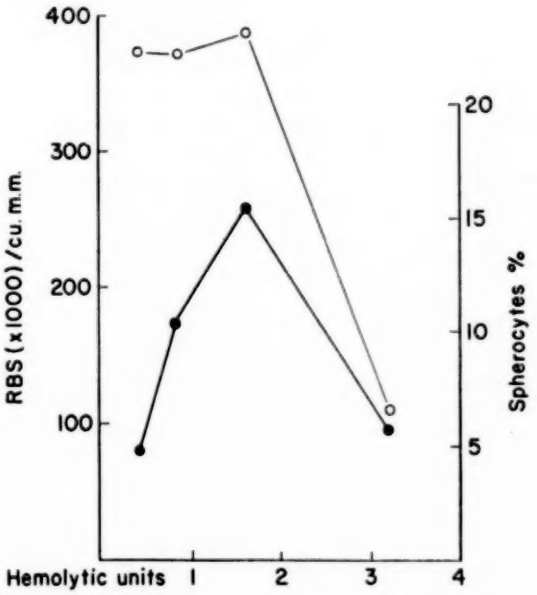

Fig. 5.—The relationship of spherocytosis to hemolysis of human red cells incubated with hemolytic fractions of spleen. Number of red cells in the mixture was taken to indicate the degree of hemolysis. As the hemolytic activity of the mixture increased and while no hemolysis was evident, the progressive rise in the concentration of hemolysin was accompanied by increased spherocytosis. As hemolysis began, the number of spherocytes dropped rapidly. Thus, spherocytic configuration preceded hemolysis.

hemolysis became severe (Fig. 5). These phenomena were prevented by the addition of adequate amounts of inhibitory fraction.

Normal red cells incubated with 3 units of hemolytic agent were totally hemolyzed in 190 minutes. Under the same experimental conditions, the cells of a patient with congenital spherocytosis were hemolyzed in 130 minutes, tion on the red cell was complex. Changes in the permeability of the red cell membrane apparently followed the incubation with subhemolytic amounts of the lysin (Table 5) with resulting loss of potassium by the erythrocyte. Also (Table 6), the incubation of red cells with a subhemolytic amount of the hemolytic fraction increased their metabolic requirement for glucose.

Rabbit erythrocytes incubated with a subhemolytic amount of the fatty acid hemolytic fraction* exhibited shortened survival time.

TABLE 5.—THE EFFECT OF SUBHEMOLYTIC AMOUNTS OF SPLENIC HEMOLYTIC FRACTION ON THE CONTENT IN SODIUM AND POTASSIUM OF HUMAN ERYTHROCYTES*

	Hematocrit %	*Na+*	*K+*
		mEq./L.	
Normal red cells	3	2.8	76.8
Cells treated with splenic fraction	2.8	5.8	28.5

*Aliquots of 5 ml. of a 10% suspension of red cells (micro-hematocrit of 6 3) were added to a subhemolytic amount of the fatty acids fraction suspended in 5 ml. of Ringer's solution. Both samples were incubated in water bath at 37° C. for 45 minutes. Micro-hematocrits were read before and after incubation. All samples were then centrifuged and the supernatant was discarded. Buttons of cells were rinsed twice with 5% solution of glucose in water and the supernatant was separated by centrifugation and discarded each time. Each button was then hemolysed by adding 2 ml. of twice distilled water and the stroma was separated by centrifugation. The concentration of potassium and sodium was then determined in the supernatant.

(e) THE ACTIVITY OF THE HEMOLYTIC FRACTION OF LEUKOCYTES AND PLATELETS. The incubation with hemolytic fractions did not affect the number, size or morphology of human platelets from normal individuals and from patients with a variety of hematological disorders. On the other hand, hemolytic fractions from the spleen containing 2 units per ml. lysed almost completely a standard suspension of white cells. By direct observation under phase contrast microscopy, the lysed ghosts appeared as a shadowy ring encircling a few peripheral granules. On stained smears, the damage to the granulocytes was evident. Intermediate stages included the effacing of the cytoplasmic granules and of the nucleus. The addition of equal amounts of inhibitory fraction from normal or from patho-

TABLE 6.—GLUCOSE CONSUMPTION OF ERYTHROCYTES AFTER EXPOSURE TO SUBHEMOLYTIC AMOUNTS OF HEMOLYTIC SPLENIC FRACTION*

	pH		*Glucose, mg./100 ml.*	
	0'	*6 hrs.*	*0'*	*6 hrs.*
Normal red cells	—	6.4	135	119
Treated red cells	—	6.3	130	91

*After separating the plasma, normal human red cells were washed once in saline and a suspension in saline containing 3 gm.% hemoglobin was prepared; 0.4 ml. were added to six tubes. Three of them contained a subhemolytic amount of the fatty acid hemolytic fraction diluted in 1 ml. of barbital buffer pH 7.5 and the other three contained only barbital buffer. All tubes were incubated in a water bath at 37° C. for 30 minutes, centrifuged and the supernatant fluid discarded. The red cell button was washed once with saline. Then 0.2 ml. of human citrated plasma and 1 ml. of 0.1% glucose solution were added to each tube. After incubation in water bath at 37° C. for 30 minutes, the pH and sugar content of the supernatant fluid in each tube was determined.

*Twenty milliliters of blood were drawn from the central artery of a rabbit's ear and transferred to a tube containing 3 ml. of ACD anticoagulation solution. After separation of plasma by centrifugation in the cold, red cells were washed 4× with saline and transferred to a test tube containing subhemolytic amount of fatty acids suspended in 9 ml. of saline. After incubation in water bath at 37° C. for one hour, 400 microcuries of $NaCr_{51}$ were added. One-half hour later, the red cells were reinjected into the donor rabbit. Samples of 1 ml. were collected 20', 60', 4 hrs. and then every other day up to 15 days after administration of tagged cells. Hematocrit and radioactivity of each sample were determined. Half life of normal rabbit red cells is 12 to 15 days with this technique. The half life of cells treated with the hemolytic fraction was 5½ days. (All these experiments were kindly contributed by Dr. Frank Gardner, Peter Bent Brigham Hospital, Boston.)

logical splenic tissue prevented this phenomenon.

Discussion. Our work confirms that a mixture of unsaturated fatty acids present in most tissues exhibits a powerful hemolytic activity. In splenic tissue, arachidonic and linoleic acid represent most of the hemolytic activity. The relative inactivity of the hemolytic fractions in normal conditions is suggested by the considerable excess of inhibitory over hemolytic fraction in all human tissues. Nevertheless, the increased susceptibility to hemolysis of the erythrocytes incubated with subhemolytic amounts of the hemolytic fraction suggests that the hemolytic system may play a role in the final destruction of aged erythrocytes, when proper conditions of stasis and damage to the red cell exist. One may be tempted to make a few speculations on the role of the hemolytic system in controlling the life span of the erythrocyte. Repeated stagnation within the tissue sinusoids may be one of the mechanisms leading to progressive chemical and enzymatic wear of the erythrocyte; in the final moments of the cell life, tissue hemolysins may contribute to the formation of the spherocyte and to its destruction.

The role of the hemolytic fractions of tissue in causing accelerated destruction of red cells may become more important in pathologic conditions and may possibly explain the increased destruction of red cells in the absence of other better understood mechanisms. In our case, unexplained hyperhemolysis was accompanied by a minimal increase of hemolytic activity and by a significant decrease in the inhibitory activity of the splenic tissue. This finding was particularly striking in patients with severe hemolytic anemia. The size of the spleen did not seem to represent an important factor in these results. The decrease of lipid fractions having hemolysis inhibiting activity seemed a constant finding in many disease states. The higher inhibitory activity of splenic tissue from patients receiving steroid therapy at adequate doses may explain the ability of steroid drugs to control the hemolytic component of many disease states.

Summary. 1. Normal spleen, brain, kidney, lung, liver, red cell stroma and serum contain fractions having lytic activity for red and white cells but not for platelets. Such activity is highest in brain, kidney, lung, liver, spleen and red cell stroma in this order. It may be due to one or more unsaturated fatty acids, arachidonic acid being possibly most important. Tissues also contain fractions with hemolysis inhibitory activity, highest in brain and spleen; lower in kidney, lung, liver and red cell stroma in that order. The inhibitory fraction is extremely heterogenous and its activity is found in many chemical fractions including proteins, cholesterol and protein-bound phospholipids and, in brain, free phospholipids.

2. Exposure to the hemolytic fraction of normal red cells cause spherocytosis, increased osmotic fragility and reduced survival time.

3. Under normal conditions, there is, in tissues, a balance between the activity of hemolysins and of inhibitory fractions. In pathologic conditions, and in splenic tissue, the hemolytic activity is increased and the inhibitory activity is strikingly decreased. This unbalance may explain severe hemolysis where no other known mechanisms are in evidence.

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SUMMARIO IN INTERLINGUA

Le Rolo, in Certe Statos Hemolytic, de un Systema Hemolytic in Tissus Human

1. Normal splen, cerebro, ren, pulmon, hepate, stroma erythrocytic, e sero contine fractiones que manifesta un activitate lytic pro erythrocytos e leucocytos sed non pro plachettas. Ille activitate es le plus forte in cerebro, ren, pulmon, hepate, splen, e stroma erythrocytic (in iste ordine). Il es possibile que illo es le effecto de un o plure non-saturate acidos grasse. Acido arachidonic es probabilemente le plus importante. Le tissus etiam contine fractiones con activitate inhibitori pro hemolyse. Isto es le plus alte in cerebro e splen e plus basse in ren, pulmon, hepate, e stroma erythrocytic (in iste ordine). Le fraction inhibitori es extrememente heterogenee; su activitate es trovate in multe fractiones chimic, incluse proteinas, cholesterol, e phospholipidos ligate a proteina o—in le caso del cerebro—phospholipidos libere.

2. Exposition al fraction hemolytic de erythrocytos normal causa spherocytosis, augmento del fragilitate osmotic, e un reducite periodo de superviventia.

3. Sub conditiones normal, il existe in le tissus un equilibrio inter le activitate de hemolysinas e del fractiones inhibitori. In conditiones pathologic (e in tissus splenic), le activitate hemolytic es augmentate, e le activitate inhibitori es reducite frappantemente. Iste disequilibrio es forsan capace a explicar sever hemolyse in casos in que nulle altere mechanismo es apparente.

VENTRICULAR PREMATURE BEATS DIAGNOSTIC OF MYOCARDIAL DISEASE

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Ventricular premature beats interrupting normal sinus rhythm is the commonest cardiac arrhythmia in man. Such beats may be asymptomatic or may produce symptoms that alarm the subject. Whether symptom producing or not, they may occur both in otherwise normal and in diseased hearts. Their mode of origin and spread of excitation cannot be determined with certainty. For these reasons, it is generally agreed that there is nothing characteristic in a premature ventricular beat which helps to determine the presence or absence of underlying myocardial disease.

On the other hand, ventricular premature beats are more common in the diseased than the normal heart. This arrhythmia should, therefore, alert one to the possibility of underlying heart disease. Preliminary observations (Soloff[16]) had suggested that premature ventricular beats arising in hearts which are the seat of structural disease are frequently different in contour from those arising in otherwise normal hearts. This work attempts to determine the relationship of the contour of premature ventricular beats to the presence or absence of underlying heart disease.

Methods and Material. A total of 6,325 electrocardiograms of 4,000 consecutive hospitalized persons were examined for the presence of premature ventricular beats. Four hundred and eleven persons had electrocardiographic evidence of premature ventricular beats. These premature beats were divided into 2 groups, 1) those with a smooth depolarization and a smooth oppositely directed repolarization which is termed the classical form, 2) those that showed variable deformities of depolarization or repolarization, or both, or those in which either J or repolarization was in the same direction as depolarization. Special care was taken to exclude superimposed P waves as the source of deformities. These two groups were then matched against clinical, radiologic or other electrocardiographic evidence of myocardial abnormality.

Results. Of the 411 subjects with premature beats, 169 had the classical pattern of ventricular premature beats. This pattern is characterized by smooth depolarization with its initial portion frequently more thickly written than its terminal segment and with smoothly written repolarization in the opposite direction. Seventy-one of these 169 occurred in otherwise normal hearts and 98 had clinical, radiologic or electrocardiographic evidence of myocardial disease. Sixty-nine of these 98 had other electrocardiographic evidence of myocardial abnormality.

The remaining 242 persons had bizarre ventricular premature beats. All had clinical, radiologic, or electrocardiographic evidence of myocardial disease. Two hundred twenty-four of the 242 had other electrocardiographic signs of myocardial disease. Of the other 18, 10 had hypertension, 4 rheumatic heart disease and the last 4 had angina pectoris, 2 of whom subsequently developed myocardial infarction.

The bizarre contours of the premature beats were of several types.

1) The duration of the QRS complex was longer than 0.18 second: there were 7, all in subjects with myocardial infarction.
2) The pattern of acute myocardial infarction: there were 13, all but one showing elsewhere the pattern of acute myocardial infarction. In this one, the premature beats anticipated the pattern of acute myocardial infarction which appeared later in ventricular beats activated by discharge of the sinus node. Evidences of an acute injury current was frequently more obvious in the premature than in the normally initiated beat.
3) Bizarre depolarization: there were 44 such instances. This pattern is characterized by irregular and uneven spread of depolarization following the expected initial slurring and after depolarization begins to be thinly written, slurring or notching again appears. These deformities may be multiple and may occur anywhere along the QRS. Distinct notching of the summit of R, as is commonly seen in bundle branch block, is included in this category. Slurring or notching may produce a distinct change in the subsequent direction of depolarization. At times, this change is so marked as to produce a shelflike contour.
4) Bizarre repolarization: there were 37 such instances excluding those showing the pattern of acute myocardial infarction. Repolarization may occur in the same direction as depolarization (5) or more commonly repolarization has an ST arch simulating that seen in coronary ischemia. Indeed, all such instances (24) occurred in subjects with other electrocardiographic evidence of coronary artery disease. Finally, depolarization may also abruptly change in direction (8) so that a shelf like appearance may also be seen.
5) Bizarre depolarization and repolarization: this was the commonest pattern (123 instances). This pattern occurred predominantly in those with other evidence of coronary artery disease (119 instances). Examples of all of these types are illustrated in Fig. 1.

Discussion. Extrasytoles occur so commonly in the otherwise normal heart and were so often at first mistaken for heart disease that Mackenzie warned that "extrasytoles in themselves are not signs of any specific injury to the heart nor should a prognosis of any gravity be based on their appearance alone"[10]. Although this statement is still in its broadest meaning correct, there is no doubt that premature ventricular (and atrial beats) have a higher incidence and greater frequency in the diseased than in the normal heart.

Bourne[3] as well as Esler and White[5] were amongst the first to attempt to interpret the clinical significance of premature beats. Bourne recognized that in the otherwise normal heart, premature beats tend to disappear with exercise. Their appearance or an increase in their number with a rising heart rate during exercise is strongly suggestive of underlying myocardial disease. White recognized that ventricular premature beats in bigeminus or in pairs after every normal beat and those which in the electrocardiogram are multiform or bidirectional are frequently evidence of a serious toxic or otherwise irritable state of the myo-

cardium. Laubry[8] as well as Porter[11] stressed the association of premature beats with angina pectoris. Premature beats on effort may be the equivalent of angina pectoris and when appearing for the first time, may like the first angina, herald the development of myocardial infarction. Later, Boas and rates and of the presence of combined atrial and ventricular premature beats. The prognostic significance of premature ventricular beats in acute coronary thrombosis is well known.

From the electrocardiographic standpoint, Scherf and Schott[13] pointed out that premature beats with wide QRS

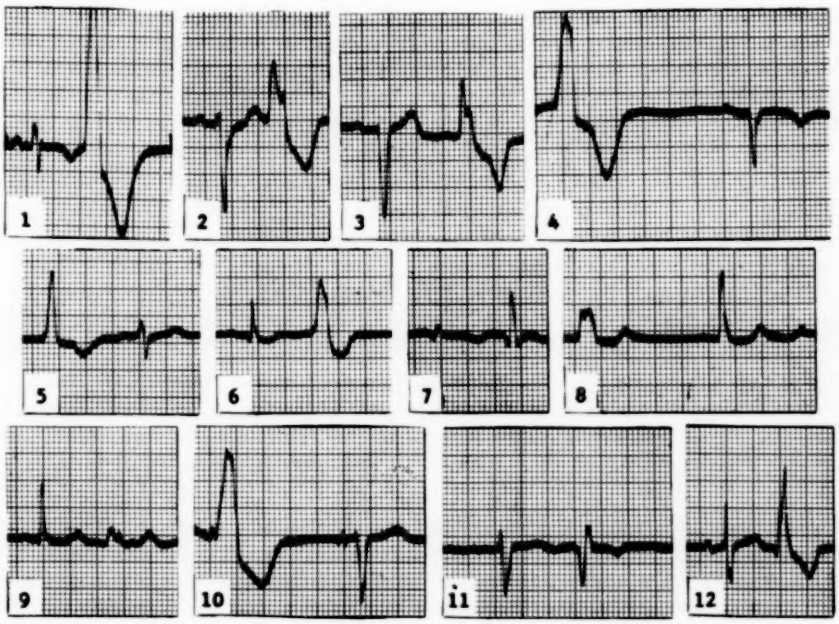

Fig. 1.—Ventricular premature beats: 1) classical type, 2) abnormal depolarization of the bundle branch block type, 3) and 4) abnormal depolarization and repolarization: note shelf-like initial repolarization particularly prominent in 5) with isolated abnormal repolarization, 6) abnormal depolarization and repolarization with concave S-T segment, 7) abnormal repolarization with S-T arched upright in same direction as QRS, 8) abnormal depolarization and repolarization with T in same direction as QRS, 9) same as 7 but note deformity due to P, 10) abnormal depolarization with wide (0.22 sec.) QRS, 11) and 12) unmasking of pattern of healed myocardial infarction.

Levy[2] discussed the clinical significance of extrasystoles. They stressed the significance of extrasystoles first appearing during or after an infection, first appearing in middle age and their presence with heart rates faster than 110 a minute. Huppert and Berliner[7] also stressed the organic significance both of atrial premature beats at rapid heart complexes tend to occur in diseased hearts. Scherf[12] also stated that inversion of an otherwise normal T wave on the first post extrasystolic beat tends to indicate structural heart disease. Smirk[15] stressed the significance of premature beats that interrupt the inscription of the preceding T wave. He felt that subjects with such electro-

cardiograms are prone to develop cardiac arrhythmia, particularly ventricular tachycardia.

Because the origin and method of spread of human ventricular premature beats cannot be determined and because these beats may occur at different phases of the refractory period of the ventricle, it is generally agreed that the form of the premature beat cannot be used to distinguish the presence or absence of underlying heart disease. Although any diseased portion of the ventricle may usurp temporarily the function of the normal pacemaker even during various phases of the refractory period, it is possible that outlying portions of the normal ventricle or conductive tissue are never bombarded and activated by such stimuli. It is also possible that a ventricular premature beat may become distorted, independent of its origin by passing through diseased tissue in a manner not unlike the distortion of a sinus activated ventricular complex that passes around a scar. Scherr and Young's[14] experimentally produced premature ventricular beats in the normal dog showed a slow conduction near the site of stimulation but normal velocity of transmission through the ventricular wall. Only an empirical analysis of premature beats in otherwise normal and those in diseased hearts can determine whether there is any significant difference between the two.

Dressler[4] was the first to demonstrate that a ventricular premature beat could unmask the pattern of acute myocardial infarction. Anttonen, Oka and Holopainen[1] have reported 39 such instances in 502 subjects with acute myocardial infarction. Herein is reported 13 instances in 411 subjects with premature ventricular beats. Equally important is the fact that 24 other subjects with coronary artery disease had final ventricular deflections typical of the coronary ischemic type. These findings indicate that premature ventricular beats that traverse an ischemic myocardium may show repolarization diagnostic of ischemia.

This study strongly suggests that deformities in depolarization of ventricular premature beats are also signs of underlying myocardial disease. In the absence of superimposed P or f waves, slurring or notching or gross changes in direction of depolarization after the initial slurred portion has been written strongly suggest that the ventricular premature beat is passing through diseased tissue. Gross[6] has stressed the wide bifid summit of premature ventricular beats as a sign of underlying heart disease. Legrand, Desruelles, Merlen, and Dubeaux[9] have verified this finding. They also reported that the lower the amplitude and the wider the ventricular complex of the premature beat, the more likely is underlying heart disease present. The findings herein reported also confirm these conclusions but indicate that a bifid summit is but one type of deformed depolarization. It is possible that a vectorcardiographic analysis of premature ventricular beats may be even more discriminatory and diagnostic.

Summary and Conclusions. 1. Electrocardiographic evidence of ventricular premature beats was present in 411 of 4,000 consecutive hospitalized subjects.

2. One hundred sixty-nine had the classical pattern and occurred in both otherwise normal and in diseased hearts.

3. Two hundred forty-two had bizarre patterns and all occurred in diseased hearts.

4. Bizarre premature ventricular beats characterized by abnormalities in depolarization, repolarization or both are highly suggestive of underlying myocardial disease.

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SUMMARIO IN INTERLINGUA

Prematur Pulsos Ventricular, Pathognomonic pro Morbo Myocardial

1. Indicios electrocardiographic de prematur pulsos ventricular esseva presente in 411 de 4.000 consecutive subjectos hospitalisate.

2. Cento sexanta-novem del subjectos habeva le classic configuration electrocardiographic. Iste serie includeva casos de cordes pathologic e etiam de cordes alteremente normal.

3. Duo centos quaranta-duo del subjectos habeva bizarre configurationes electrocardiographic. In omne iste casos il se tractava de cordes pathologic.

4. Bizarre prematur pulsos ventricular—characterisate per anormalitates de depolarisation, de repolarisation, o de ambes—es altemente suspecte de subjacente morbo myocardial.

THE ACTION OF CERTAIN ANTIBIOTICS AND ANTIBIOTIC COMBINATIONS AGAINST *PROTEUS MIRABILIS* WITH DEMONSTRATION OF IN VITRO SYNERGISM AND ANTAGONISM*

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Proteus species are often implicated in chronic urinary tract infections (Kass[10]) and occasionally cause serious systemic disease such as bacteremia or meningitis (Abrams[1], Rosove, West and Bower[13], Spittel *et al.*[16]). In recent years there has been an apparent increase in the incidence of infections caused by these microorganisms (Chiu and Hoeprich[3], Hanson, Karabatsos and Herrold[6], Potee, Wright and Finland[12]). Finland has reported that at the Boston City Hospital the number of cases of Proteus septicemia per year steadily increased during the period 1941 to 1957 and that Proteus species were the most common cause of death from bacteremia caused by Gram-negative bacilli in 1955 and 1957 (Finland, Jones and Barnes[5]).

Infections due to Proteus species present a difficult therapeutic problem because of the general insusceptibility of these microorganisms to antimicrobial agents (Chiu and Hoeprich[3], Hanson, Karabatsos and Herrold[6], Potee, Wright and Finland[12]). The present investigation was undertaken in order to gain more information about the action of antibiotics against *Proteus mirabilis*, the species which constitutes about 75% of Proteus isolates (Eisenberg, Weiss and Flippin[4], Hanson, Karabatsos and Herrold[6], Hook and Petersdorf[7], Potee, Wright and Finland[12]). In these studies the susceptibility of 25 strains of *Pr. mirabilis* to certain antibiotics and antibiotic combinations was determined. In addition, the rate of antimicrobial action against one strain was studied in detail.

Materials and Methods. SOURCE OF STRAINS. The strains of Proteus included in this study were isolated from patients at The New York Hospital during March and April, 1960. Thirty consecutive strains from different patients with clinical and laboratory evidence of significant Proteus infections were selected for further bacteriologic testing. Three strains were isolated from blood, 13 from urine collected by catheterization of the urinary bladder or ureter, and 14 from midstream urine specimens. When voided urine was the source, patients with less than 100,000 bacteria per ml. were eliminated from the study. All 30 patients had urinary tract dis-

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ease with chronic pyelonephritis as the most frequent disease entity, and most had been previously treated with several antibiotics.

CHARACTERISTICS OF STRAINS. All 30 strains of Proteus were Gram-negative rods that swarmed on blood agar when incubated for 24 hours at 37° C. All isolates failed to ferment lactose but did produce urease within 48 hours when grown in Urease Test Medium obtained from Baltimore Biological Laboratory. Twenty-seven of the 30 Proteus strains were identified as *Pr. mirabilis* according to the criteria of Rustigian and Stuart[15], that is, failure to ferment maltose, and inability to produce indol when incubated in 1% Bacto-Tryptone (Difco) for 48 hours and then tested with Kovac's reagent.

ANTIBIOTIC SENSITIVITIES. Twenty-five of the 27 strains of *Pr. mirabilis* were tested against potassium penicillin G, chloramphenicol, dihydrostreptomycin sulfate* and kanamycin sulfate. Each antibiotic was diluted in 2-fold steps in 12 × 100 mm. test tubes containing 0.5 ml. of beef-heart infusion broth. The bacterial inoculum for each tube was 0.5 ml. of a 10^{-4} dilution of an 18-hour broth culture of the strain to be tested. The bacteriostatic end-point was considered to be the minimum concentration of antibiotic that prevented turbidity in the test tube after 24 hours of incubation at 37° C. From each tube without visible growth, about 0.005 ml. was removed with a sterile platinum loop and streaked on a blood-agar plate. After 24 hours incubation at 37° C. the plates were examined and the lowest concentration of antibiotic which resulted in no growth was taken to be the bactericidal end-point. The highest concentration of antibiotic used in the sensitivity tests was 50 units per ml. of penicillin or 50 μg. per ml. of each of the other 3 antibiotics.

Each strain was also tested against the following mixtures of drugs: penicillin with chloramphenicol, dihydrostreptomycin or kanamycin, and chloramphenicol with kanamycin. The highest concentration of antibiotic used in each combination was 25 units per ml. of penicillin or 25 μg. per ml. of each of the other 3 antibiotics. Serial two-fold dilutions were made from these combinations.

STUDIES OF RATE OF ANTIBIOTIC ACTION. The rates of action of penicillin, dihydrostreptomycin, kanamycin and chloramphenicol, alone or in certain combinations, were determined using the Botero strain of *Pr. mirabilis* which had been originally isolated from the blood of a patient. The antibiotic sensitivity of this microorganism is shown in Table 1. Flasks containing varying concentrations of antibiotics or antibiotic combinations in infusion broth at 37° C. were inoculated with an appropriate aliquot of an 18-hour culture of the Botero strain to give a 10^{-4} dilution of the inoculum. Aliquots were taken from each flask at intervals of time and diluted in 100-fold steps in broth at 4° C. Pour plates were made using a 1 ml. aliquot of each dilution of test solution, 10 ml. of melted trypticase soy agar, and 100 units of penicillinase (Schen Labs) in 0.1 ml. of sterile distilled water. The agar was allowed to harden and then a second layer of agar was poured to prevent swarming. The plates were incubated at 37° C. for 24 hours and bacterial colonies counted on plates containing 10 to 1,000 colonies.

Aliquots were periodically removed from the flasks for determination of concentrations of antibiotics and for isolation of organisms for antibiotic sensitivity tests. The concentrations of antibiotics were determined, after Seitz filtration, by microbiological assay using a microorganism highly sensitive to each antibiotic.

The strains of Proteus were kept on agar slants at 4° C. and were transplanted monthly.

Results. ACTIVITY OF SINGLE ANTIBIOTICS. In the range of antibiotic concentrations tested, kanamycin was the most effective single drug (Fig. 1). The 25 strains of *Pr. mirabilis* were inhibited by 25 μg. per ml. of kanamycin and this level was bactericidal for 22 strains. Penicillin in a concentration of 25 units per ml. was bacteriostatic for 17 strains and bactericidal for 6, and dihydrostreptomycin in a concentration of 25 μg. per ml. was bacteriostatic for 13 and bactericidal for 12 strains. Although 25 μg. per ml. of chloramphenicol inhibited 20 of the 25 strains, this concentration of drug was lethal for only 5 strains.

Kanamycin or dihydrostreptomycin usually killed at concentrations of antibiotic that were the same or twice the inhibiting levels. With penicillin

*Although dihydrostreptomycin is the antibiotic standard routinely used in this laboratory, sensitivities to streptomycin have been found to be identical.

TABLE 1.—ANTIBIOTIC SENSITIVITY OF THE BOTERO STRAIN OF *PROTEUS MIRABILIS*

	Antibiotic Concentration per ml.	
	Bacteriostatic	*Bactericidal*
Penicillin	6.25 units	25 units
Chloramphenicol	12.5 μg.	>50 μg.
Dihydrostreptomycin	3.13 μg.	6.25 μg.
Kanamycin	12.5 μg.	25 μg.
Penicillin and Kanamycin	1.56 units and 1.56 μg.	6.25 units and 6.25 μg.
Penicillin and Dihydrostreptomycin	1.56 units and 1.56 μg.	3.13 units and 3.13 μg.
Penicillin and Chloramphenicol	3.13 units and 3.13 μg.	> 25 units and 25 μg.
Chloramphenicol and Kanamycin	3.13 μg. and 3.13 μg.	25 μg. and 25 μg.

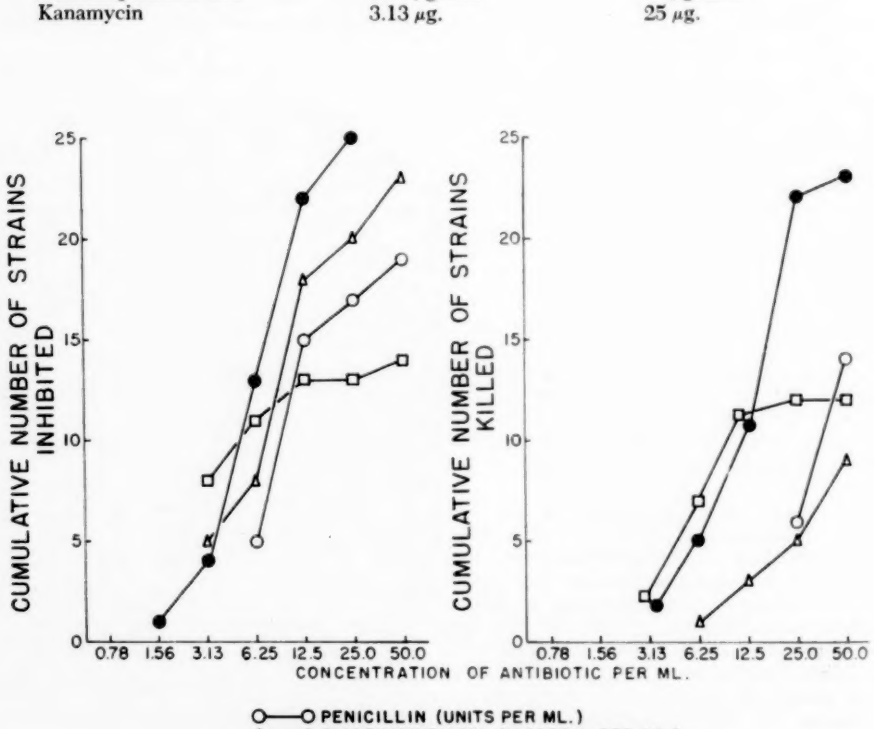

Fig. 1.—Susceptibility of 25 strains of *Pr. mirabilis* to the action of 4 antibiotics.

or chloramphenicol there was usually no bactericidal effect until levels at least 4 times the inhibiting concentration were reached.

ACTIVITY OF COMBINATIONS OF ANTIBIOTICS. In this paper, additive bacteriostatic effect is defined as inhibition of growth of a microorganism *in vitro* by a combination of antimicrobial drugs containing no more than one-half the minimal inhibitory concentration of each drug. The sensitivity of each strain of Proteus to single antibiotics and antibiotic mixtures has not been included because of the large volume of data involved. Instead, as shown in Figs. 2 to 5, the susceptibilities of the 25 Proteus strains to single antimicrobials and mixtures of antimicrobials have been expressed as cumulative number of strains affected by increasing concentrations of drug.

The combination of penicillin with kanamycin demonstrated an additive bacteriostatic effect for most of the Proteus strains. As shown in Fig. 2, all 25 strains were inhibited by a combination of 12.5 units of penicillin and 12.5 μg. of kanamycin per ml. and 24 strains were inhibited by 6.25 units of penicillin and 6.25 μg. of kanamycin per ml. This combination showed a striking increase in bactericidal effect. Microorganisms were usually killed by a combination containing only one-eighth to one-fourth of the bactericidal concentration of the more effective drug. All strains were killed by a combination of 12.5 units of penicillin with 12.5 μg. of kanamycin per ml. (Fig. 2).

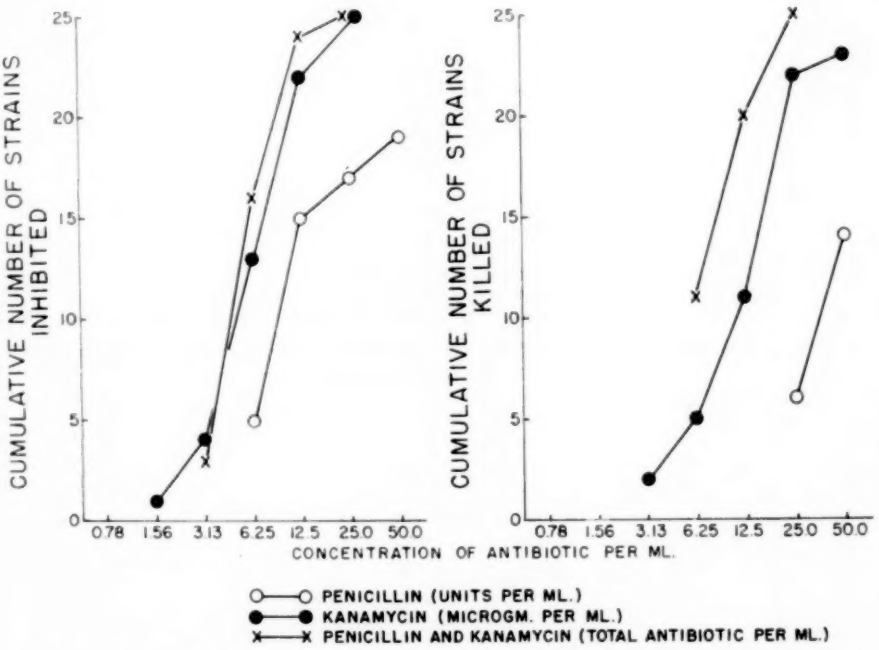

Fig. 2.—Susceptibility of 25 strains of *Pr. mirabilis* to the action of a combination of penicillin and kanamycin.

The combination of penicillin with dihydrostreptomycin (Fig. 3) demonstrated an additive inhibitory effect for the majority of strains sensitive to di-

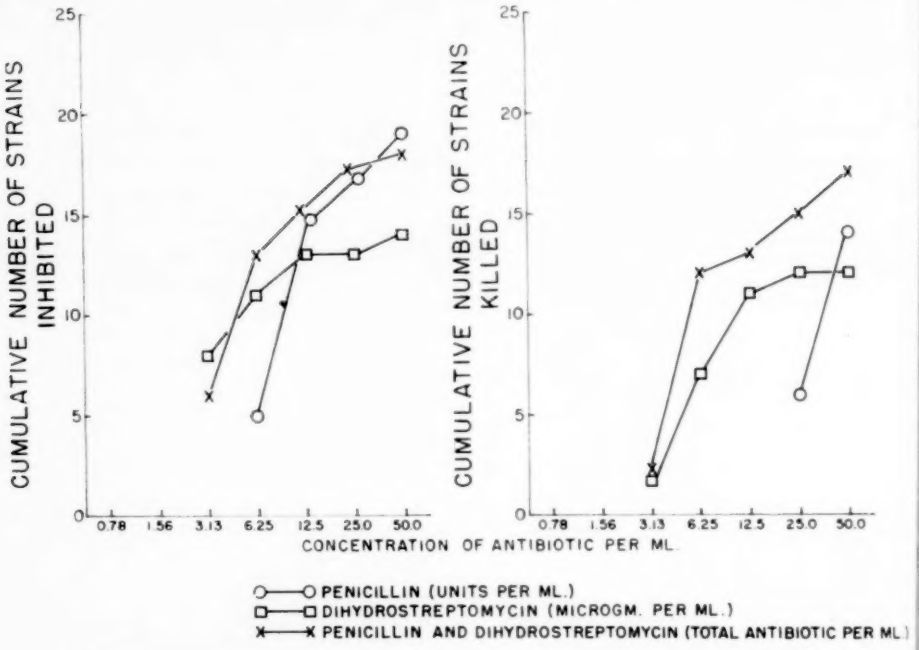

Fig. 3.—Susceptibility of 25 strains of *Pr. mirabilis* to the action of a combination of penicillin and dihydrostreptomycin.

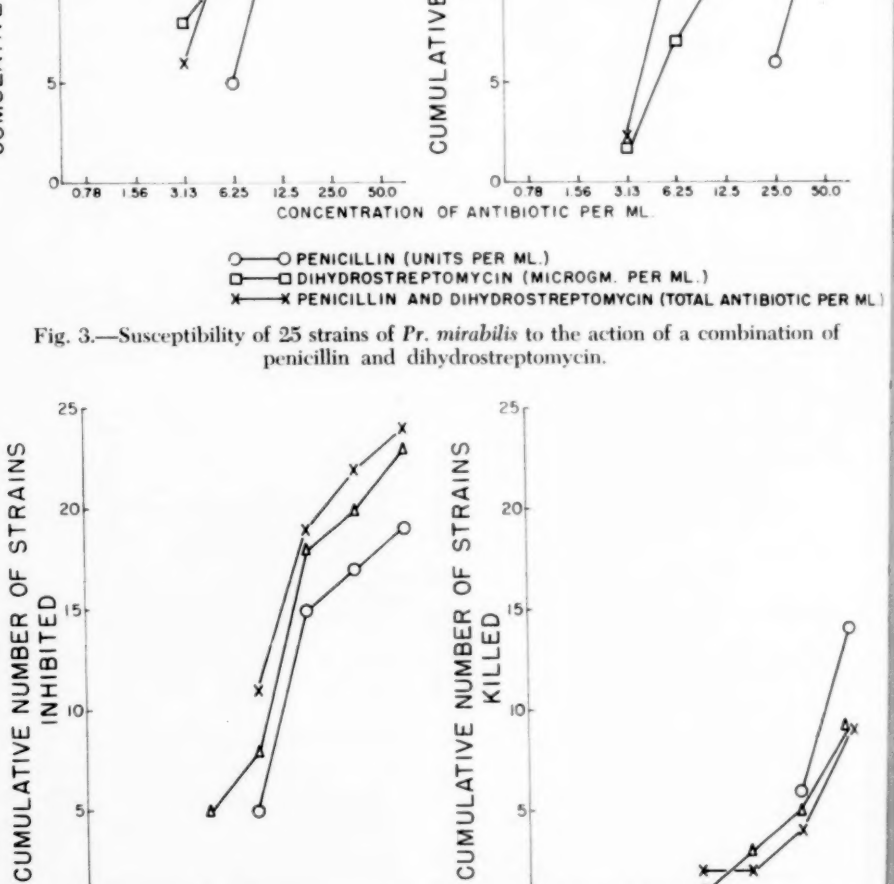

Fig. 4.—Susceptibilty of 25 strains of *Pr. mirabilis* to the action of a combination of penicillin and chloramphenicol.

hydrostreptomycin. Although an increase in bactericidal activity was noted against most dihydrostreptomycin-sensitive strains, the increased effect was striking in only one-third of these strains.

The combinations of chloramphenicol with penicillin (Fig. 4) or chloramphenicol with kanamycin (Fig. 5) had an additive bacteriostatic effect for

RATE OF ANTIBIOTIC ACTION. In order to study the rate of antimicrobial activity, single antibiotics or combinations of drugs were mixed with standard inocula of the Botero strain of *Pr. mirabilis* in flasks, and aliquots were removed at intervals for enumeration of bacteria (see **Materials and Methods**). As shown in Figs. 6 and 7, 20 units of penicillin, 20 μg. of kanamycin,

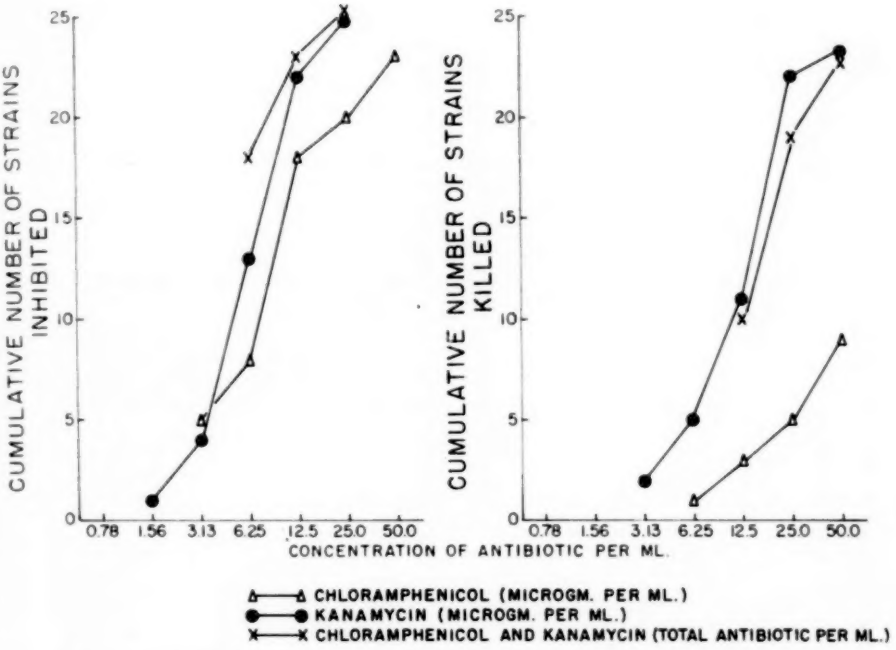

Fig. 5.—Susceptibility of 25 strains of *Pr. mirabilis* to the action of a combination of chloramphenicol and kanamycin.

about one-half of the strains. The growth of 22 strains was inhibited by the combination of 12.5 units of penicillin with 12.5 μg. of chloramphenicol per ml., while all 25 strains were inhibited by the combination of 12.5 μg. of chloramphenicol with 12.5 μg. of kanamycin per ml. There was no increase in bactericidal effect with these combinations. In fact there seemed to be a suggestion of decreased effect.

or 10 μg. of dihydrostreptomycin per ml. resulted in 100-1000 fold decrease in the number of culturable microorganisms after 6-hours' incubation. However, these concentrations of drugs were not sufficient to eliminate Proteus from the flasks and bacterial multiplication occurred after 6 to 24 hours. Proteus cultured from each flask after 6 to 12 hours of incubation was tested and found to be significantly more re-

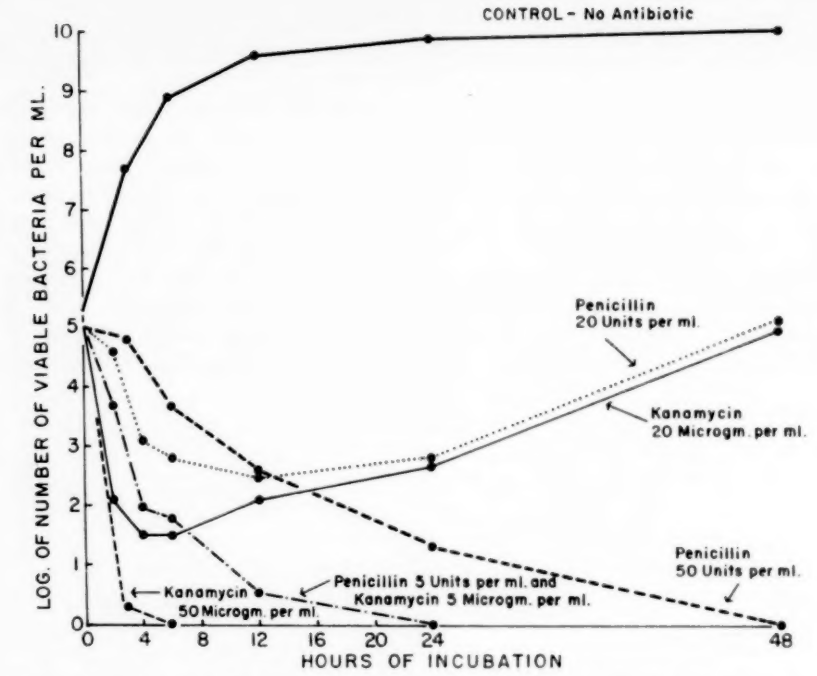

Fig. 6.—The action of penicillin, kanamycin and a combination of these drugs on the Botero strain of Proteus.

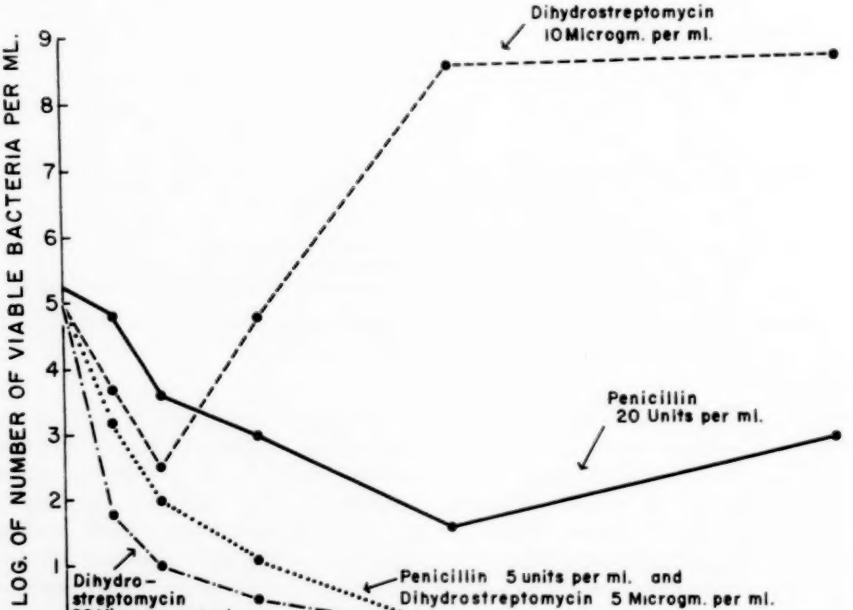

Fig. 7.—The action of penicillin, dihydrostreptomycin and a combination of these drugs on the Botero strain of Proteus.

sistant to the antibiotic in the medium than the original culture.

Complete sterilization of the inoculum could be achieved by increasing the concentration of penicillin, dihydrostreptomycin or kanamycin. Dihydrostreptomycin in a concentration of 20 μg. per ml. (Fig. 7) or kanamycin in a concentration of 50 μg. per ml. (Fig. 6) resulted in a rapid decrease in the number of culturable organisms and a sterile 1 ml. aliquot at 24 and 6 hours respectively. Fifty units per ml. of penicillin (Fig. 6) caused a slower rate of decrease of bacteria and required 48 hours for sterilization of a 1-ml. aliquot.

In the experiments described in the preceding paragraphs, it was shown that penicillin in a concentration of 50 units per ml., kanamycin in a concentration of 20 μg. per ml., or dihydrostreptomycin in a concentration of 10 μg. per ml. did not eliminate Proteus from the flask in 24 hours. However, when a combination of 5 units of penicillin and 5 μg. of either kanamycin or dihydrostreptomycin per ml. was added to the flask, microorganisms could not be cultured from a 1 ml. aliquot at 24 hours (Fig. 6 and 7). Chloramphenicol (Fig. 8) in a concentration of 50 μg. per ml. effectively inhibited multiplication but did not significantly reduce the bacterial titer during the initial 24 hours. The combination of chloramphenicol with penicillin or kanamycin showed decreased activity when compared to the same concentration of penicillin or kanamycin alone. The de-

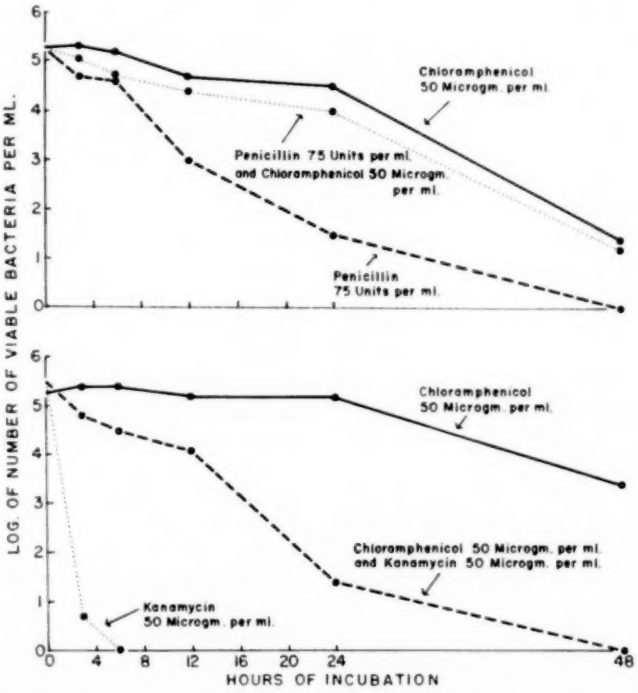
LOG. OF NUMBER OF VIABLE BACTERIA PER ML.
HOURS OF INCUBATION
Chloramphenicol 50 Microgm. per ml.
Penicillin 75 Units per ml. and Chloramphenicol 50 Microgm. per ml.
Penicillin 75 Units per ml.
Chloramphenicol 50 Microgm. per ml.
Chloramphenicol 50 Microgm. per ml. and Kanamycin 50 Microgm. per ml.
Kanamycin 50 Microgm. per ml.

Fig. 8.—The action of chloramphenicol and the combinations of chloramphenicol with penicillin and chloramphenicol with kanamycin on the Botero strain of Proteus.

creased effect of the combination was usually most apparent during the early killing phase of 3 to 6 hours for kanamycin and 12 to 24 hours for penicillin (Fig. 8).

Although the levels of chloramphenicol, dihydrostreptomycin and kanamycin did not decrease significantly* during the initial 24 hours of incubation, the activity of penicillin at 24 hours was always less than one-half of the initial concentration.

Discussion. The 90% incidence of *Pr. mirabilis* among Proteus strains isolated was even higher than expected but certainly supports previous observations that this species makes up the vast majority of Proteus strains found in clinical material (Eisenberg, Weiss and Flippin[4], Hanson, Karabatsos and Herrold[6], Hook and Petersdorf[7], Potee, Wright and Finland[12]).

On the basis of the *in vitro* observations recorded in this study, kanamycin was the most effective single drug against *Pr. mirabilis*. Although penicillin was only a moderately good bacteriostatic and bactericidal agent at a concentration of 50 units per ml., it has been shown that levels of penicillin higher than those tested here are effective against a greater proportion of *Pr. mirabilis* strains (Chiu and Hoeprich[3], Hook and Petersdorf[7], Potee, Wright and Finland[12]). In judging relative merit of different antimicrobials, it should be noted that penicillin is a relatively nontoxic drug and that levels well over 50 units per ml. are attainable *in vivo*. In contrast, *in vivo* levels of over 22 μg. of kanamycin, 40 μg. of streptomycin, or 12.5 μg. of chloramphenicol per ml. are difficult to maintain (Welch[18]). Furthermore, hearing loss and serious nephrotoxicity have been reported with use of kanamycin (Waisbren *et al.*[17]), fatal blood dyscrasias have occurred in patients treated with chloramphenicol[2], and impairment of vestibular function and deafness have occasionally followed use of streptomycin (Rossi *et al.*[14]). Susceptibilities to the tetracycline drugs were not determined because of previous work demonstrating that strains of *Pr. mirabilis* are almost always highly resistant to these drugs (Eisenberg, Weiss and Flippin[4], Hook and Petersdorf[7], Poole[11], Potee, Wright and Finland[12]).

The bactericidal activity of the penicillin-kanamycin combination, as determined by the antibiotic dilution method on 25 strains of *Pr. mirabilis*, was greatly increased over either single drug and suggested that a synergistic effect occurred against most strains. The antibiotic dilution results also seemed to indicate that the combination of penicillin with dihydrostreptomycin was synergistic against some of the dihydrostreptomycin-sensitive strains. In contrast, observations on the chloramphenicol combinations suggested drug antagonism for a significant proportion of strains. According to Jawetz and Gunnison[8], *in vitro* antibiotic synergism and antagonism should be expressed as a function of rate of action of the antibiotics. For this reason, detailed studies were performed on one strain (Botero) of *Pr. mirabilis* to determine the actual rate of antimicrobial action.

Dihydrostreptomycin or kanamycin exerted a definite bactericidal effect against the Botero strain of *Pr. mirabilis* as shown by a marked rapid decrease in the number of viable organisms (Figs. 6 to 8). In contrast, chloramphenicol yielded essentially no decrease in culturable bacteria during a 24-hour period (Fig. 8) and was, therefore, bacteriostatic. Penicillin was between these two extremes in activity

*The microbiological assay used will not detect changes in antibiotic concentration of less than a two-fold magnitude.

but was more like dihydrostreptomycin and kanamycin.

Combinations of penicillin with kanamycin or dihydrostreptomycin provided a marked increase in the rate of bactericidal action against the Botero strain exceeding that obtained by 2 to 4 times the concentration of either drug alone. According to Jawetz *et al.*[8,9], this is an example of *in vitro* synergism, which he defines as a marked increase in the rate of early bactericidal action of an antibiotic mixture beyond that obtainable by simple additive effects of the agents. Combinations of chloramphenicol with penicillin or kanamycin caused a marked reduction in the rate of early bactericidal action against the Botero strain as compared to the more effective drug alone and, therefore, according to Jawetz and Gunnison[8] is an example of *in vitro* drug antagonism. They define antagonism as a marked decrease in the rate of early bactericidal action of an antibiotic combination below that observed with the single more active drug.

As demonstrated in Figs. 6 and 7, nonsterilizing concentrations of kanamycin, dihydrostreptomycin or penicillin killed all but a fraction of the microorganisms before multiplication occurred. It is apparent from these observations that tube-dilution "bacteriostatic" results for these 3 drugs were a function of rate and degree of *killing* and rate of multiplication of persisters. Only chloramphenicol exhibited a truly bacteriostatic action. Furthermore, it should be noted that the bactericidal endpoint as determined by the conventional antibiotic dilution method is arbitrary as only 0.005 ml. of culture media is subcultured. Therefore, if fewer than 200 viable microorganisms per ml. are present, the subculture will probably be sterile. This may help explain the apparent discrepancies between sensitivities of the Botero strain as determined by the antibiotic dilution method (Table 1) and the results of the studies demonstrated in Figs. 6 to 8 where 1 ml. aliquots were subcultured.

While it is difficult to apply the results of *in vitro* studies to clinical situations, it would seem reasonable to explore the use of high doses of penicillin together with kanamycin or streptomycin (when the infecting organism is sensitive to the latter drug) in the treatment of serious infections caused by *Proteus mirabilis*. Streptomycin and not dihydrostreptomycin should be used because of the greater risk of loss of auditory function associated with administration of the latter drug (Rossi *et al.*[14]).

Summary. Twenty-seven of 30 strains of Proteus isolated from patients with clinical and laboratory evidence of significant Proteus infections proved to be *Proteus mirabilis*. Twenty-five strains of *Proteus mirabilis* were tested against several different antibiotics and antibiotic combinations. Kanamycin was the most effective bacteriostatic and bactericidal drug. All combinations tested showed an additive inhibitory effect most marked with penicillin and kanamycin. This combination also demonstrated a striking increase in bactericidal activity against the majority of strains. Penicillin plus dihydrostreptomycin showed a striking increase in bactericidal effect for only one-third of the dihydrostreptomycin-sensitive strains. Chloramphenicol combined with penicillin or kanamycin showed no advantage in bactericidal activity over the more effective component alone.

Studies on the rate of antibiotic action against one strain demonstrated synergism between penicillin and kanamycin or dihydrostreptomycin and antagonism between chloramphenicol and penicillin or kanamycin.

ACKNOWLEDGMENT: Miss Pepi Romano rendered valuable technical assistance.

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SUMMARIO IN INTERLINGUA

Le Action de Certe Antibioticos e Combinationes de Antibioticos Anti *Proteus mirabilis*, con Demonstration in Vitro de Synergismos e Antagonismos

Vinti-septe de 30 racias de Proteus, isolate ab patientes con indicationes clinic e laboratorial de infection per Proteus, esseva identificate como *Proteus mirabilis*. Vinti-cinque racias de *Proteus mirabilis* esseva testate contra plure differente antibioticos e combinationes de antibioticos. Kanamycina esseva le droga le plus efficace como bacteriostatico e como bactericida. Omne le combinationes testate monstrava un additive effecto inhibitori, le plus marcatemente com penicillina e kanamycina. Iste combination etiam produceva un frappante augmento del activitate bactericida contra le majoritate del racias. Penicillina in combination con dihydrostreptomycina monstrava un frappante augmento del effecto bactericida pro solmente un tertio del dihydrostreptomycino-sensibile racias. Chloramphenicol combinate con penicillina o kanamycina monstrava nulle avantage in activitate bactericida in comparation con le componente plus efficace sol.

Studios del intensitate del action antibiotic anti un del racias revelava synergismo inter penicillina e kanamycina o dihydrostreptomycina e antagonismo inter chloramphenicol e penicillina o kanamycina.

THE APPLICATION OF THE SENSITIZED SHEEP CELL TEST TO SERA OF PATIENTS WITH NONRHEUMATIC DISEASES AND FALSE POSITIVE SEROLOGICAL REACTIONS FOR RHEUMATOID ARTHRITIS*

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There are now available several serological procedures for use in the laboratory diagnosis of rheumatoid arthritis. These reactions all presumably depend on the presence in the serum of a high molecular weight protein, or proteins, contained in the globulin fraction that has been termed the "rheumatoid factor." The unusual affinity of the rheumatoid factor for normal gamma globulin forms the basis for the agglutinating activity.

The factor has been recognized in most, but not all, of the individuals with rheumatoid arthritis. Likewise, it has been identified in seemingly unrelated, nonrheumatic diseases which are accompanied by altered plasma proteins. An increasing number of reports have appeared, detailing positive serum agglutination reactions in patients with abnormal serum globulins using the FH techniques (the latex, Bentonite and tanned sheep cell tests) but with no overt evidence of rheumatic disease (deForrest, Mucci and Boisvert[2], Dresner and Trombly[3], Howell, Malcolm and Pike[9], Kunkel, Simon and Fudenberg[10], Peltier and Christian[12]). These tests are positive in 70 to 95% of patients with clinical rheumatoid arthritis, depending on the stage of the disease and the procedure employed (Rheins *et al.*[13], Ziff[17]). In control studies on the serum of normal persons, 1 to 3% have been reported as reactive (Ziff[17]).

In 1956, Rheins *et al.*[13] reported positive latex fixation tests in 7 of 24 patients with syphilis, and in 1957, Ziff reported a 16% positive incidence in a group of patients with hepatitis[17]. Since that time, there has appeared a number of reports of positive reactions in patients, who, during the course of nonrheumatic diseases, usually had an abnormality of their serum proteins (Dresner and Trombly[3], Howell, Malcolm and Pike[9]).

The disease most frequently associated with a false positive rheumatoid test is viral hepatitis. Positive reactions have been obtained in 16 to 30% of

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these patients, with the test often becoming negative with remission of the disease process (Dresner and Twombly[3], Howell, Malcolm and Pike[9], Ziff[17]). Recently, Powell, Malcolm and Pike reported an incidence of 32.6% positive rheumatoid factor in a series of 43 patients with liver disease; the majority of the patients had acute viral hepatitis or portal cirrhosis[9].

The purpose of this study is to compare the results of the several currently used serological tests for rheumatoid arthritis, in patients with abnormal serum globulins, in order to determine if any degree of specificity could be elicited for the test employed.

Materials and Methods. PATIENT POOL. A total of 105 patients, 66 male and 39 female, formed the basis of this study. Their ages ranged from 16 to 73 years, with an average age of 40 years. The patients had diagnoses of Laennec's cirrhosis, sarcoidosis, syphilis, hyperglobulinemia of unknown etiology, lymphopathia venereum, hyperglobulinemic purpura (Waldenström), macroglobulinemia (Waldenström), multiple myeloma, and cryoglobulinemia.

PORTAL CIRRHOSIS. There were 28 patients studied; all had a clinical diagnosis of portal cirrhosis, which, in most cases, was confirmed by needle biopsy. The total serum protein ranged from 5.2 to 10.0 gm.%, with an average of 7.68 gm.%. The average total globulin was 4.08, with a range of 2.5 to 6.2 gm.%.

SARCOIDOSIS. Eleven patients with a diagnosis of sarcoidosis had typical roentgenological findings in the lungs or bone, hyperglobulinemia and occasionally hypercalcemia or inflammation of the uveal tract. The total serum protein in this group of patients ranged from 8.0 to 9.2 with an average of 8.5 gm.%. The average globulin content was 5.8 with a range of 5.1 to 6.9 gm.%.

SYPHILIS. There were 9 patients with a diagnosis of syphilis. The diagnosis was made on the findings of repeated positive serological tests for syphilis with or without a clinical history compatible with the disease. The total protein ranged from 7.3 to 8.8, with an average of 8.0 gm.%; the average globulin fraction was 4.7 gm.%.*

HYPERGLOBULINEMIA. Elevated globulins were observed in 7 individuals without any demonstrable cause. The total serum protein ranged from 6.6 to 6.8, with an average of 6.7 gm.%. The total globulin fractions ranged from 4.1 to 4.5, with an average of 4.2 gm.%.

LYMPHOPATHIA VENEREUM. In 2 patients, this diagnosis was made from the presence of typical inguinal or rectal lesions together with a positive Frei skin test. The total protein was 8.1 and 9.0 gm.% and the globulin fractions were 5.3 and 5.7 gm.%, respectively.

HYPERGLOBULINEMIC PURPURA (WALDENSTRÖM). There were 5 patients studied and the diagnosis was based on the criteria outlined in Waldenström's original report (Waldenström and Winblad[16]). This syndrome consists of benign, recurrent, nonthrombocytopenic purpura, elevated erythrocyte sedimentation rate, and diffuse hypergammaglobulinemia with no demonstrable underlying disease. Macroglobulinemia and cryoglobulinemia were excluded from this group of patients. The total protein ranged from 8.1 gm.% to 9.2 gm.%, with a mean of 8.75 gm.%; the globulin fraction ranged from 3.6 gm.% to 6.1 gm.% with a mean of 5.0 gm.%.

MACROGLOBULINEMIA (WALDENSTRÖM). There were 12 patients studied with this diagnosis which was based upon the ultracentrifuge finding of a large amount of macroglobulin (16S or higher) in the serum, a homogenous narrow peak on paper electrophoresis and the characteristic increase in serum viscosity in the cold. Clinically, the patients were quite variable but were usually elderly males with anemia and asthenia. The total proteins ranged from 5.4 gm.% to 12.0 gm.% with a mean of 7.92 gm.%. The globulin ranged from 2.3 gm.% to 7.8 gm.% with a mean of 4.63 gm.%.

MULTIPLE MYELOMA. There were 27 patients diagnosed clinically as multiple myeloma on the finding of skeletal lesions, the appearance of the typical sharp peak in the serum or urine paper electrophoretic pattern, and bone marrow or necropsy findings of plasma cell infiltration. The total protein ranged from 3.9 gm.% to 11.0 gm.% with a mean of 8.28 gm.%. The globulin ranged from 1.3 gm.% to 10.9 gm.% with a mean of 4.88 gm.%.

CRYOGLOBULINEMIA. The diagnosis in these 4 patients was made by finding large amounts of gamma globulin precipitated from serum kept at 4° C. for 12 hours. A small amount of cryoprecipitate may occur in many disease states and these were excluded. Although

*The total protein and globulin contents were determined in only 7 sera.

cryoglobulins are most frequently encountered in multiple myeloma, they were found in one patient with lymphosarcoma and no etiology was discovered in the other 2 patients. The total protein ranged from 7.5 gm.% to 8.7 gm.% with a mean of 8.1 gm.%. The globulin ranged from 3.4 gm.% to 4.1 gm.% with a mean of 3.8 gm.%.

Whole blood was drawn from the antecubital vein and allowed to clot. The serum obtained was then stored at −15° C. and all of the tests were performed immediately after the sample was thawed.

this procedure, suspended particles of Wyoming Bentonite clay are obtained by selective gravity for uniform size. These particles were sensitized with Fraction I, lyophilized human gamma globulin, and incorporated in a two-fold serial dilution drop slide flocculation method. This test was interpreted as positive if, microscopically, flocculation of 2 plus or more occurred at dilutions of 1:32 or greater.

The sensitized sheep cell agglutination (SSC) test was carried out on whole serum employing the procedure described by Heller,

TABLE 1.—NUMBERS OF POSITIVE REACTORS IN ALL GROUPS OF PATIENTS USING THE WHOLE SERUM LATEX FIXATION TEST

	Number Tested			*Number Positive*		
	Sex			*Sex*		
Disease	*M*	*F*	*Total*	*M*	*F*	*Total*
Laennec's cirrhosis	16	12	28	3	2	5
Sarcoidosis	8	3	11	4	0	4
Syphilis	5	4	9	0	0	0
Hyperglobulinemia of unknown etiology	3	4	7	0	0	0
Lymphopathia venereum	2	0	2	0	0	0
Hyperglobulinemic purpura (Waldenström)*	1	4	5	1	4	5
Macroglobulinemia of Waldenström*	8	4	12	1	2	3
Multiple Myeloma*	19	8	27	0	0	0
Cryoglobulinemia	4	0	4	1	0	1
Totals for series	66	39	105	10	8	18

*A portion of these patients have been included in another study[4].

The latex fixation test was performed according to the method of Singer and Plotz[15]. Each test serum, except that specimen with the pyroglobulin, was inactivated at 56° C. for 30 minutes to decrease the incidence of the prozone phenomenon (Schubart, Cohen and Calkins[14]). Latex particle size was 0.8140 microns. Fraction II, lyophilized human gamma globulin†, served as the sensitizing agent. Whole serum (1:20) was tested in serial two-fold dilutions. Tests were considered diagnostically positive if agglutination occurred at dilutions of 1:20 or greater. A positive and a negative control were included with the tests. Agglutination was considered to be present if the supernatant was relatively clear after centrifuging and agglutinated particles could be identified with a hand lens after gentle agitation.

The Bentonite flocculation test was carried out as described by Bozicevich *et al.*[1]. In

Jacobson and Kolodny[6]. Serum is twice absorbed with washed, packed sheep erythrocytes to remove heterophil antibody. It is then inactivated at 56° C. for 30 minutes and tested with 3% sheep erythrocytes which have been sensitized with anti-sheep hemolysin. The reaction mixture is then read for agglutination. The test was considered positive if agglutination occurred at serum dilutions of 1:28 or greater.

Results. The results of the latex fixation procedure, using whole serum, are summarized in Table 1. Of the 28 patients with an established diagnosis of portal cirrhosis, 5 were positive reactors. Four patients with sarcoidosis had positive tests. Five patients with hyperglobulinemia of Waldenström and

†Courtesy of the American Red Cross.

3 patients with macroglobulinemia had positive tests. Negative results were found in the 5 patients with syphilis, the 7 patients with hyperglobulinemia of unknown etiology, the 2 individuals with lymphopathia venereum, the 27 patients with multiple myeloma, and the 3 with cryoglobulinemia.

The results using the Bentonite flocculation test are summarized in Table 2. In this series, positive reactions were obtained in 7 of the 28 patients with portal cirrhosis, 5 with sarcoidosis, 3 of the 9 individuals with a diagnosis of cirrhosis, sarcoidosis, multiple myeloma, syphilis, idiopathic hyperglobulinemia, and lymphopathic venereum, had negative reactions. In macroglobulinemia, it is interesting to note that there was an association between the positive reactors and the patient's sex; all the males had positive tests, while all the females had negative tests.

Total serum proteins with A/G ratios were performed on most of the sera. We were unable to correlate positive tests with either an increase in the total serum protein or total globulin. The highest total protein and globulin values were obtained in patients with negative serological tests.

TABLE 2.—NUMBER OF POSITIVE REACTORS IN ALL GROUPS USING THE BENTONITE FLOCCULATION TEST

	Number Tested			*Number Positive*		
	Sex			*Sex*		
Disease	*M*	*F*	*Total*	*M*	*F*	*Total*
Laennec's cirrhosis	16	12	28	2	5	7
Sarcoidosis	8	3	11	4	1	5
Syphilis	5	4	9	2	1	3
Hyperglobulinemia of unknown etiology	3	4	7	0	2	2
Lymphopathia venereum	2	0	2	0	0	0
Hyperglobulinemic purpura	1	4	5	1	4	5
Macroglobulinemia	8	4	12	2	3	5
Multiple myeloma	19	8	27	0	0	0
Cryoglobulinemia	3	0	3	0	0	0
Total	65	39	104	11	16	27

The results of the sensitized sheep cell agglutination test are summarized in Table 3. A positive reaction was observed in 4 of the 5 patients with hyperglobulinemic purpura, 5 of the 12 patients with macroglobulinemia, and one individual with a diagnosis of cryoglobulinemia. The patients with portal syphilis, 2 of the 7 patients with hyperglobulinemia of unknown etiology, all of the individuals with hyperglobulinemic purpura, and 5 of the individuals with macroglobulinemia. As with the latex fixation test, no positive reactors to the Bentonite flocculation test were found in the patients with lymphopathia venereum, multiple myeloma, and cryoglobulinemia.

Discussion. It has been increasingly obvious that several of the gamma globulin fractions react in a manner similar to the rheumatoid factor when the F II procedures are employed. Kunkel, Simon and Fudenberg extensively studied a group of patients with positive serologic reactions for the rheumatoid factor in the hyperglobulinemic states[10]. There were 61 patients with sarcoidosis, of whom 6 had a positive latex reaction in high titer. The sera showed unusually high total protein concentrations and distinctly increased gamma globulins. The serum of one of

these individuals revealed a 22S complex on ultracentrifugation. This serum, however, was consistently negative when tested with the SSC even though a positive latex fixation test was found in a titer of magnitude rarely observed in rheumatoid arthritis. These authors also observed 2 patients with cirrhosis who had positive latex and SSC tests but no evidence of arthritis.

Although our series contains no patients with viral hepatitis, the results we obtained in patients with cirrhosis would seem to confirm the finding of Howell, Malcolm and Pike[9]. These authors found that the incidence of positive reactions in F II agglutination tests is higher in patients with parenchymatous liver involvement than in other patients with abnormal serum globulins.

TABLE 3.—NUMBER OF POSITIVE REACTORS IN ALL GROUPS USING THE SENSITIZED SHEEP CELL AGGLUTINATION TEST

	Number Tested			*Number Positive*		
	Sex			*Sex*		
Disease	*M*	*F*	*Total*	*M*	*F*	*Total*
Laennec's cirrhosis	16	11	27	0	0	0
Sarcoidosis	7	3	10	0	0	0
Syphilis	5	4	9	0	0	0
Hyperglobulinemia of unknown etiology	3	4	7	0	0	0
Lymphopathia venereum	2	0	2	0	0	0
Hyperglobulinemic purpura	1	4	5	1	3	4
Macroglobulinemia	8	4	12	5	0	5
Multiple myeloma	19	8	27	0	0	0
Cryoglobulinemia	4	0	4	1	0	1
Total	65	38	103	7	3	10

Lospalluto, Lewis, and Ziff separated the agglutinating factor from the sera of patients with rheumatoid arthritis; ion exchange chromatography yielded two distinct purified macroglobulins, only one of which reacted in the SSC test[11]. Heimer, Federico and Freyberg absorbed an agglutinating factor from serum of rheumatoid arthritis on sheep cell stroma[5]. The supernate obtained was found to contain at least 3 macroglobulins which were reactive in the tanned sheep cell test and were distinguishable from the macroglobulin retained on the stroma. Howell, Malcolm and Pike conjectured that the macroglobulin adsorbed on the stroma of sheep cells was more specific for rheumatoid arthritis[9].

Waldenström and Winblad found that sera of patients with hyperglobulinemic purpura gave as many positive SSC reactions as did the sera of patients with rheumatoid arthritis[16]. They pointed out that joint symptoms, especially arthralgia, occurred in a few of these individuals with hyperglobulinemic purpura but clinical polyarthritis was not observed. The finding of 4 positive SSC reactors in 5 patients with hyperglobulinemic purpura, and 5 of the 12 individuals with a diagnosis of macroglobulinemia, one of which had an exceptionally high titer, is of interest and would seem to warrant further study (Hammack and Holley[4]). There was no clinical evidence of rheumatoid arthritis in these patients.

In view of the increased positive SSC test in macroglobulinemia and hyperglobulinemic purpura of Waldenström, it might be postulated that these disorders might represent a "variant" of the "collagen diseases" (Hammack and Holley[4]). Certainly the entire spectrum of these diseases has not been clearly delineated. These disorders have in common an elevated gamma globulin and an increase in tissue lymphocytes and plasma cells. Pathologically, the syndromes are described as chronic inflammatory or chronic granulomatous disorders. The plasma proteins responsible for the positive reactions in these states appear also to be in the gamma fraction, but as yet have not been definitely characterized.

Summary. Serum specimens from 105 patients with hyperglobulinemia were examined by the sensitized sheep cell test, latex fixation test and Bentonite flocculation test. These patients had diagnoses of Laënnec's cirrhosis, sarcoidosis, syphilis, hyperglobulinemia of unknown etiology, lymphopathia venereum, hyperglobulinemic purpura of Waldenström, macroglobulinemia of Waldenström, multiple myeloma and cryoglobulinemia. The latex fixation procedure, using whole serum, resulted in 5 positive reactions in Laënnec's cirrhosis, 4 in sarcoidosis, 5 in hyperglobulinemia of Waldenström, and 3 with macroglobulinemia. Negative results were found in syphilis, hyperglobulinemia of unknown etiology, lymphopathia venereum, multiple myeloma and cryoglobulinemia. The Bentonite flocculation test resulted in positive reactions in 7 patients with portal cirrhosis, 5 with sarcoidosis, 3 with syphilis, 2 with hyperglobulinemia of unknown etiology, 5 with hyperglobulinemic purpura of Waldenström, and 5 with macroglobulinemia. No positive reactions were found in lymphopathia venereum, multiple myeloma and cryoglobulinemia. The sensitized sheep cell agglutination test gave positive reactions in 4 patients with hyperglobulinemic purpura, 5 patients with macroglobulinemia and 1 with cryoglobulinemia. Negative results were obtained with portal cirrhosis, sarcoidosis, multiple myeloma, syphilis, idiopathic hyperglobulinemia and lymphopathia venereum. It is interesting to note that in macroglobulinemia, all males had positive sensitized sheep cell tests, while all females had negative results. This study suggests that the sensitized sheep cell agglutination test possesses a higher degree of specificity for the laboratory diagnosis of rheumatoid arthritis than the FII agglutination procedure. The finding of positive sensitized sheep cell agglutination reactors among patients with hyperglobulinemic purpura and macroglobulinemia are interesting observations and may reveal a basic similarity of these disorders.

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SUMMARIO IN INTERLINGUA

Le Application del Test con Sensibilisate Cellulas Ovin a Seros de Patientes con Morbos Non-Rheumatic e con Falsemente Positive Reactiones pro Arthritis Rheumatoide

Specimens de sero ab 105 patientes con hyperglobulinemia esseva examinate per medio del test a sensibilisate cellulas ovin, del test de fixation a latex, e del test de flocculation a Bentonite. Iste patientes habeva diagnoses de cirrhosis de Laënnec, sarcoidosis, syphilis, hyperglobulinemia sin cognoscite etiologia, lymphopathia veneree, hyperglobulinemic purpura de Waldenström, macroglobulinemia de Waldenström, myeloma multiple, e cryoglobulinemia. Le methodo del fixation a latex—con le uso de sero total—resultava in 5 reactiones positive in cirrhosis de Laënnec, 4 in sarcoidosis, 5 in hyperglobulinemia de Waldenström, e 3 in macroglobulinemia. Resultatos negative esseva incontrate in syphilis, hyperglobulinemia sin cognoscite etiologia, lymphopathia veneree, myeloma multiple, e cryoglobulinemia. Le test de flocculation a Bentonite resultava in reactiones positive in 7 patientes con cirrhosis portal, 5 con sarcoidosis, 3 con syphilis, 2 con hyperglobulinemia sin cognoscite etiologia, 5 con hyperglobulinemic purpura de Waldenström, e 5 con macroglobulinemia. Nulle reactiones positive esseva incontrate in lymphopathia veneree, myeloma multiple, e cryoglobulinemia. Le test del sensibilisate cellulas ovin produceva reactiones positive in 4 patientes con hyperglobulinemic purpura, 5 con macroglobulinemia, e 1 con cryoglobulinemia. Resultatos negative esseva obtenite con cirrhosis portal, sarcoidosis, myeloma multiple, syphilis, hyperglobulinemia idiopathic, e lymphopathia veneree. Es interessante notar que in macroglobulinemia omne le masculos habeva positive reactiones in le test de agglutination de sensibilisate cellulas ovin, durante que omne le femininas habeva resultatos negative. Iste studio pare indicar que le test del agglutination de sensibilisate cellulas ovin possede un plus alte grado de specificitate pro le diagnose laboratorial de arthritis rheumatoide que le technicas de agglutination a factor II (i.e., le test a latex, le test a Bentonite, e le test a tannite cellulas ovin). Le constatation de positive reactores (in le test de agglutination a sensibilisate cellulas ovin) inter le patientes con purpura hyperglobulinemic e macroglobulinemia es interessante in tanto que illo signala possibilemente un similitude fundamental inter iste disordines.

THE EFFECT OF PHYSICAL EXERCISE ON SERUM TRANSAMINASE*

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with the technical assistance of

C. A. Kirkpatrick, HM1, USN

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A patient with chest pain following strenuous physical exercise failed to conform to the expected clinical picture of myocardial infarction, although the serum transaminase was elevated. The experience prompted a study of the effect of physical exercise on the serum glutamic-oxalacetic transaminase (SGOT) and the serum glutamic-pyruvic transaminase (SGPT) in healthy males.

Methods. SGOT and SGPT were determined by the method of Karman, Wroblewski and LaDue[3] modified by Reitman and Frankel[4]. The upper limit of normal in this laboratory is taken to be 40 units for SGOT and 45 units for SGPT.

The subjects were male Marine Corps and Naval Personnel ranging in age from 17 to 34 years. The average age for the group was 21 years. The usual occupations of the participants varied with their service ratings. The exercises recorded in the table were either part of a physical fitness testing program or were voluntarily undertaken for the purpose of the present study. There was no obvious disease in any of the subjects. The exercise in each instance was strenuous according to the classification offered by Yater and associates[7] who consider strenuous such activities as drilling, marching, running, laboring and participation in sports. One individual whose control (preexercise) SGOT and SGPT were 56 and 80 units respectively was excluded from the study.

Blood was drawn from an antecubital vein after the application of a tourniquet which was left in place during the bleeding.

Results. The results are tabulated in Table 1 and shown in the scattergram (Fig. 1). It will be seen that in the swimmers, control levels are not given. It was felt undesirable to expose the subjects to even the hypothetical risk of infection that might attend venipuncture immediately prior to immersion in a swimming pool.

The data indicate that there is a nearly uniform, moderate rise in the level of SGOT immediately after exercise and that the serum level of the enzyme returns to normal within 12 to 16 hours. SGPT shows a much smaller rise which tends to remain within the normal range.

Discussion. At the beginning of this study, no report on the effect of exercise on serum transaminase levels had appeared in the available English literature, although such an effect had been observed by Wroblewski during the course of some studies[5]. While the paper was in preparation, a report appeared which mentioned, but did not detail, a rise in SGOT with no rise in SGPT following physical exercise (Henley, Schmidt and Schmidt[2]).

Glutamic-oxalacetic and glutamic-pyruvic transaminases are present in several tissues. Skeltal muscle and cardiac muscle are particularly rich in SGOT (Wroblewski and LaDue[6]). The mechanism whereby exercise of skeletal muscle raises the level of SGOT is unknown. Injury in the usual sense would seem to play no part. The rise

*The opinions given are those of the author and are not necessarily those of the Naval Service at large.

in SGOT following exercise, complicates the interpretation of elevated levels of the enzyme in the serum of patients with chest pain following strenuous physical exertion. In such a patient, the elevated SGOT could result from the exercise *per se* or result from a myocardial infarction induced by strenuous exercise or indeed, result from a combination of both.

The direct role of exercise in raising the level of the SGOT in this clinical setting may be assessed to a degree by applying the criteria that in exercise induced elevations, the SGPT is not raised above the normal level; the SGOT has not thus far exceeded 100 units and the elevated level returns to normal within 12 to 16 hours. Although SGPT may be within normal limits and

TABLE 1.—SGOT AND SGPT BEFORE AND AFTER EXERCISE

			SGOT			*SGPT*		
Subject	*Age*	*Physical Activity*	*Before*	*Immed. After*	*12–16 Hrs. After*	*Before*	*Immed. After*	*12–16 Hrs. Aft.*
1	20	Chin-ups, etc. and ½	30	60	30	24	40	24
2	34	mile jogtrot	32	62	30	26	45	28
3	31		32	60	30	26	38	28
4	28		28	52	26	24	32	22
5	19	Running for 5 minutes	30	58	30	21	34	21
6	19	(jogtrot)	30	53	27	23	30	20
7	22	Running 0.9 mi. in 7½ min.	27	58	29	20	30	22
8	17	Swim 150 yds. around	*	53	**	*	34	**
9	18	pool	*	69	**	*	34	**
10	18		*	55	**	*	30	**
11	17		*	63	**	*	40	**
12	18		*	63	**	*	40	**
13	17		*	58	27	*	37	18
14	17		*	44	27	*	26	22
15	23		*	48	27	*	26	20
16	18		*	58	26	*	30	22
17	20		*	48	24	*	26	20
18	18		*	44	24	*	26	18
19	21	Rowing 1 5/6 mi. in	24	38	26	20	28	18
20	21	racing shell	26	44	26	22	27	20
21	22		26	54	23	20	30	18
22	22		34	48	30	22	40	22
23	22		28	48	24	20	28	17
24	25		22	36	**	18	26	**
25	21		28	62	28	20	36	18
26	21		42	96	45	28	47	26
27	19	Coxswain of shell	34	42	24	20	28	20

*See explanation in text.
**Failed to return for follow-up.

SGOT be less than 100 units in myocardial infarction, the elevated SGOT tends not to return to normal within 12 to 16 hours, but rather reaches peak values in 18 to 36 hours (Conrad[1]).

Summary. A study was made of the effect of physical exercise on the serum transaminase. It was demonstrated that physical exercise of a moderately strenuous nature regularly raises the level of the serum glutamic-oxalacetic transaminase (in most instances to levels above the upper limit of normal). The serum glutamic-pyruvic transaminase, although showing a slight rise, tends to remain within normal limits.

Since the transaminase level is so frequently used in the laboratory diagnosis of myocardial infarction, and since infarction occasionally appears to be precipitated by exercise, confusion in interpretation of transaminase levels after exercise may arise. The finding that the elevated level of transaminase induced by exercise returns to normal within 12 to 16 hours permits a resolution of such a diagnostic problem.

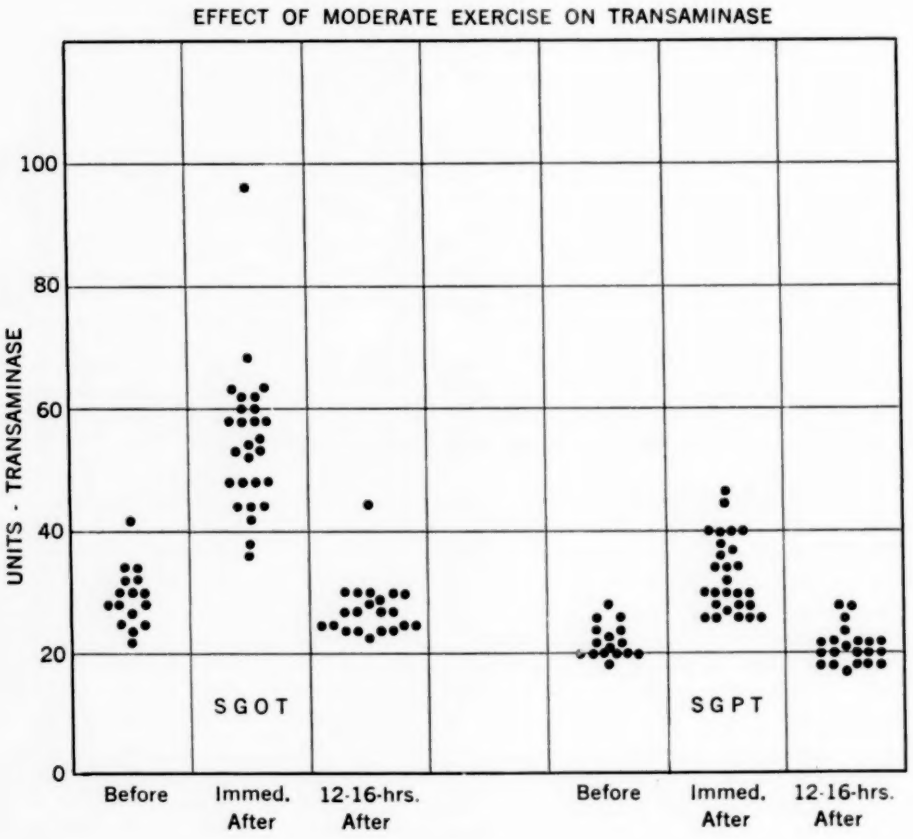

Fig. 1.

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SUMMARIO IN INTERLINGUA

Le Effecto de Exercitios Physic Super le Transaminase del Sero

Esseva studiate le effecto de exercitios physic super le transaminase del sero. Esseva demonstrate que exercitio physic de character moderatemente sever augmenta regularmente le nivello del transaminase glutamic-oxaloacetic del sero (in le majoritate del casos a nivellos supra le limite superior del region normal). Le nivello seral de transaminase glutamic-pyruvic, ben que illo monstra un leve augmento, tende a remaner intra le region normal.

Viste que le nivellos de transaminase es frequentemente usate in le diagnose laboratorial de infarcimento myocardial, e viste etiam que infarcimento pare occasionalmente esser precipitate per exercitio, il es obvie que confusion pote resultar del interpretation de nivellos de transaminase post exercitio. Iste problema in le diagnose pote esser resolvite a base del observation que le elevation inducite in le nivellos de transaminase per exercitios physic non persiste in ultra de 12 a 16 horas.

COOMASSIE BLUE USED IN THE ESTIMATION OF PLASMA VOLUME: A PRELIMINARY REPORT

COMPARISON OF THE DYE WITH OTHER INDICATORS AND A REPORT OF VALUES OBTAINED IN 10 NORMAL ADULT SUBJECTS*

By William Bradley Martin†

(From the Duluth Cardiovascular and Surgical Research Laboratory, St. Mary's Hospital, Duluth, Minnesota, Josiah Fuller, M.D., Director)

The volume of plasma can be determined by injecting into the blood a suitable quantity of an indicator and measuring its concentration when mixing is complete. The relationship between the value desired and the two ascertainable factors is shown in the formula:

Plasma volume, expressed in liters = $\frac{\text{quantity of indicator injected, expressed in milligrams}}{\text{concentration of the indicator, expressed in milligrams per liter of plasma.}}$

This may be abbreviated to:

$$\text{P.V.} = \frac{\text{I}}{\text{C}}$$

where P.V. represents plasma volume, I the amount injected and C the concentration of the indicator at the time of complete mixing.

The ideal indicator is 1) so nontoxic that repeated injections can be made in a single day, since clinicians find comparative tests most useful. 2) The ideal indicator mixes uniformly in the plasma. 3) It is inert, not combining with any substance existing outside the plasma. 4) It does not leave the circulation until mixing is complete. 5) It persists long enough to permit withdrawal of at least one, and preferably two, blood samples for the determination of plasma concentration. 6) The indicator does not persist in the plasma so long as to make repeated determinations difficult, or repeated injections hazardous. 7) The behavior of the indicator is well enough known and sufficiently consistent to permit selection of a time for complete mixing. 8) The indicator's properties are of such a nature that its concentration in the plasma can be determined accurately, not being affected by hemolysis or lipemia.

All indicators may be discussed under these headings and their virtues and disadvantages tabulated. For example, Iodine$_{131}$-labeled albumin meets most of the criteria, but persists overly long and multiple determinations might exceed safe radiation limits. Evans blue dye is long persistent and repeated injections carry the risk of skin discoloration. Indocyanine dye (Cardio-Green) (Fox *et al.*[3]) has certain characteristics that make it useful as an indicator in cardiac diagnosis, but its brief persistence in plasma makes it unsuitable for volume determinations (Cherrick *et al.*[1]).

Iodine-labeled albumin mixes uniformly in the plasma, acting in this respect as native albumin; the azo-dyes stain the native albumin. Thus considerable similarity in the behavior of these two indicators may be anticipated. Both are assumed to go where

*Supported in part by a Minnesota Heart Association Grant.
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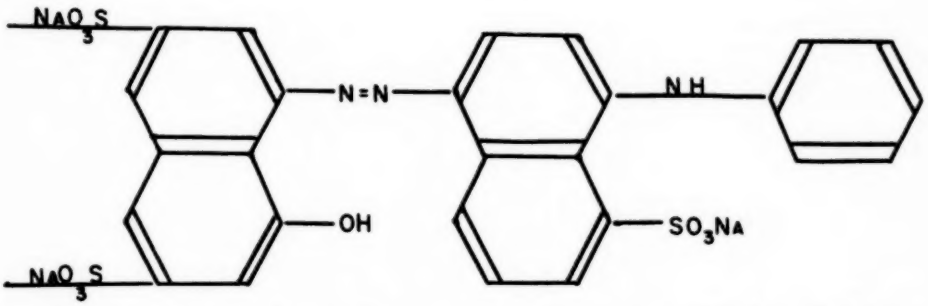
albumin goes and not elsewhere. These substances should be called "indicators of albumin space;" but this is a function of plasma volume if not identical to it.

THE INDICATOR DYE. Coomassie Blue R.L. is a purified monoazo dye synthesized by Imperial Chemical Industries, Ltd., England, and made available in the United States by Ayerst Laboratories, New York.* The sodium salt of the dye is soluble in water, stable in storage and may be autoclaved. The structural formula is given by the manufacturer as follows:



The color is due to the azo linkage, (–N=N–), intensified by the balance between the rings on either side. The sulfate groups contribute the solubility. The dye is packaged as a 2% solution in 5 ml. ampules containing 20 mg. per ml. and 100 mg. per ampule.

The dye was proven nontoxic in mice, rats, rabbits and dogs in a conventional toxicity investigation (Taylor and Thorp[9]). Its use in humans was tested by Taylor and Shillingford[8] and later by Oakley and associates[7]. Taylor and Shillingford injected 15 to 150 mg. into 93 patients ranging in age from 3 years to adulthood. Oakley *et al.* used doses of 10 to 70 mg. in 69 patients; some individuals received 10 separate injections. The latter investigators stated that toxic effects in humans ". . . have been negligible, consisting only in occasional vomiting, usually after more than 5 to 6 injections. The vomitus was inky blue due to excretion of dye in the bile. Skin discoloration was absent or minimal and rapidly disappeared since there is complete clearance of the dye from the body in 12 hours." The authors mentioned receiving reports from other investigators of toxic reactions following Coomassie Blue injections consisting of vaso-vagal symptoms and vomiting in instances where doses of over 500 mg. had been given. The dye's nontoxicity in normal humans was convincingly shown by Kalz and Fekete[6] who administered 400 mg. of Coomassie Blue in a single injection to 125 volunteers without ill effect.

As regards the *in vivo* stability of the dye, Taylor and Shillingford[8] reported no loss when circulated once through muscle, lung or liver. Its inertness was further demonstrated by nearly complete recovery of the dye after being mixed with blood *in vitro*. The rate of disappearance of Coomassie Blue *in vivo* will be considered under **Results.**

Coomassie Blue is measured in a spectrophotometer at its point of maximum light absorption, 585 millimicrons wave length. Optical densities and concentration are linearily related and the relationship does not vary with different plasmas. The extraction procedure

*Richard A. Carey, M.D., Ayerst Laboratories, supplied the dye.

eliminates spectrophotometric error due to hemolysis or lipemia.

Procedure. Ten normal adult male volunteers were tested. The ages, measurements, packed cell volumes, corrected packed cell volumes and percents of plasma of these subjects are shown in Table 1. They were not in a basal state. With the patient comfortably reclining, an ear-piece oximeter* was positioned on the pinna after preliminary warming of the ear and the ear-piece. The photoelectric currents from the ear-piece transducer were led to a logarithmic amplifier† and the output recorded by a Heiland visicorder.‡ The arterial concentration curve data will be the subject of a future report. The ear-piece readings were useful in predicting the time when complete mixing occurred.

With a tourniquet applied to the subject's arm, hanging dependent over the side of the cot, a venous blood sample was withdrawn for use as a dye-free blank in the photometric determination and for the construction of a graph relating optical density to concentration. Using a weighed syringe, the dye, varying in amount from 40 to 90 mg., was injected into a forearm vein as rapidly as possible at the instant of tourniquet release. The previously dependent arm was abruptly elevated within one second of the time of injection. This abrupt injection of the indicator and ensuing arm elevation to flush the dye into the circulation are necessary for satisfactory arterial concentration curves but are not necessary for plasma volume determinations. In all instances two or more venous blood samples were withdrawn from the opposite arm at various intervals after injection, for example: 30 seconds, 1 to 30 minutes and 1 to 24 hours.

TABLE 1.—AGE, PHYSICAL MEASUREMENTS, PACKED CELL VOLUME, CORRECTED PACKED CELL VOLUME AND PERCENT OF PLASMA IN 10 NORMAL ADULT MALE SUBJECTS

Case No.	*Age years*	*Height cm.*	*Weight kg.*	*[a]S.A. m^2*	*[b]P.C.V. ·100*	*[c]Corrected P.C.V.*	*% plasma 100–(PCV · 91)*
(1)	*(2)*	*(3)*	*(4)*	*(5)*	*(6)*	*(7)*	*(8)*
1	42	182.9	78.9	2.01	42.5	38.7	61.3
2	26	175.3	86.2	2.02	47.5	43.2	56.8
3	27	171.5	63.5	1.75	44.5	40.5	59.5
4	31	170.2	73.5	1.84	44.5	40.5	59.5
6	30	163.8	65.8	1.71	46.0	41.9	58.1
7	35	179.1	83.0	2.03	45.5	41.4	58.6
8	25	172.7	76.6	1.90	45.0	41.0	59.0
9	37	171.5	78.5	1.92	40.0	36.4	63.6
12	34	166.4	68.0	1.77	49.0	44.6	55.4
13	39	186.7	83.0	2.09	41.5	37.8	62.2

a = Surface area (ref. 2)
b = Packed Cell Volume ("Hematocrit")
c = Corrected P.C.V. =PCV · 91 (ref. 5)

The blood samples were expelled from the collecting syringe into graduated tubes previously moistened with 4 drops of a heparin solution containing 1,000 units per ml. After repeated gentle inversions the blood samples were centrifuged** at 3,000 revolutions per minute for 30 minutes. The hematocrit values were read, the plasma fraction removed and the dye extracted following the method of Taylor and Shillingford[8]. The optical density of the samples was then measured against known concentration of dye in a spectrophotometer†† at 585 millimicrons. The dye con-

*As devised by Earl H. Wood, M.D., Rochester, Minnesota, and available from Waters Corporation, Rochester, Minnesota.

†Developed by Curt A. Wiederhielm and marketed by Engineering Specialty Company, Salt Lake City, Utah.

‡Available from Minneapolis-Honeywell-Heiland Division, Denver, Colorado.

**International Centrifuge, size 1, Model SBV. Head No. 240.
International Equipment Co., Boston Massachusetts.

††Junior Spectrophotometer. Coleman Instrument Inc., Maywood, Illinois.

centration of the venous blood samples was recorded in milligrams per liter of plasma and a disappearance curve of dye was plotted.

Results. TOXICITY. No side effects were noticed by the volunteers receiving the dye injection, with one exception. In this individual, the dye was inadvertently injected outside the vein. Rather quickly a bluish discolored area appeared measuring 4 by 8 cm. centering at the site of injection. During the next 24 hours this area was moderately painful, quite tender and slightly warm to touch. The discoloration slowly disappeared in 4 days. The local heat and tenderness vanished within 2 days. Ulceration or permanent pigmentation did not occur. Six individuals received 2 or 3 injections of 60 mg. of dye within an hour for replicate determinations of cardiac output; in these individuals there were no ill effects observed.

MIXING OF THE DYE AND DISAPPEARANCE CONSTANT. Some dye was detected in venous samples within one minute of injection. The concentrations rose to a peak at 2 to 3 minutes and then declined abruptly. The plots of dye concentration against elapsed time beginning at 3 minutes and continuing through 5, 10, 15, 30, 60 and 120 minutes consisted of two distinct components. The first, a rapid descent, the writer believes represents mixing of the dye. This process terminates at 10 or 15 minutes after injection, as the

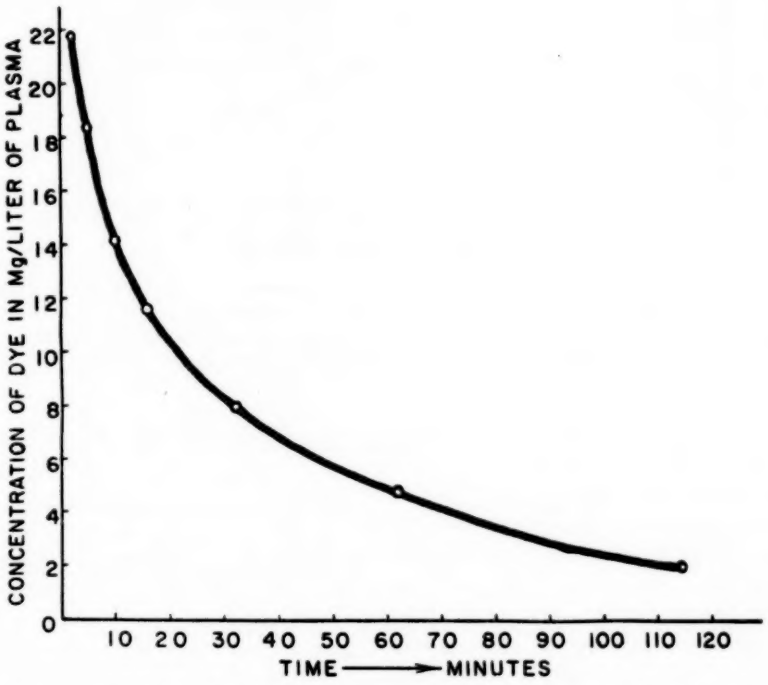


Fig. 1.—A linear plot of results in experimental case No. 14, a normal adult male, weighing 99 kg. Concentrations existing in plasma, expressed in milligrams per liter of plasma are plotted against elapsed time. Zero time in this graph is the instant of injection of 81 mg. of dye in the antecubital vein.

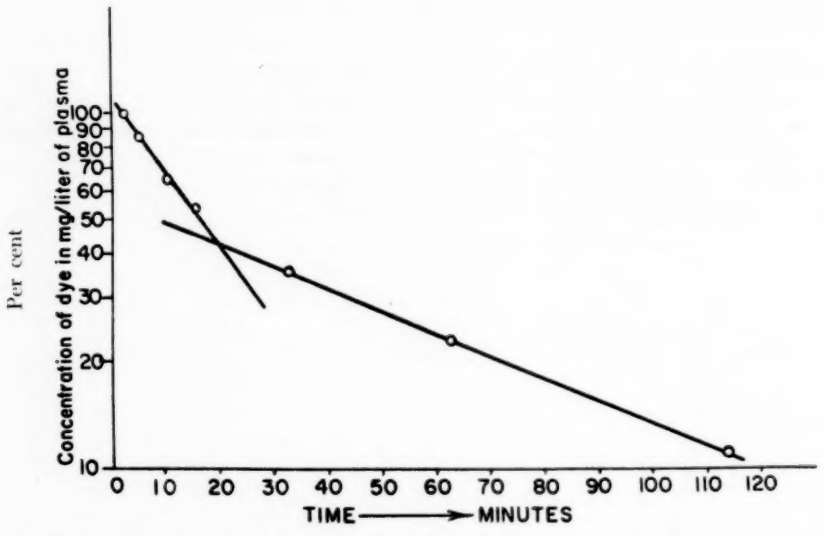


Fig. 2.—The same data used for Fig. 1 have been plotted on semi-logarithmic paper. The 2-minute concentration was placed equal to 100% and ensuing concentrations were computed as a percentage of this to ascertain ordinate values. These figures are displayed against elapsed time on the linear scale of the abscissa.

curves from then on slope downward at a slower rate as an exponential function constant for different individuals.

Figure 2 shows the same data as in Fig. 1, this time plotted on semi-logarithmic paper. Two distinct straight lines can be recognized. In this instance they meet 18 minutes after injection, although as remarked, the junction of the two lines was more frequently at 10 or 15 minutes after injection.

Figure 1 shows the plasma concentrations of Coomassie Blue plotted against time using the data obtained in a normal adult male, weighing 99 kg. who received 81 mg. of dye. He was not a member of the original group. The plasma concentration curves of the experimental group were similar but a larger number of points were available in the subject of the graph.

The formula for plasma decay is:

$$C_{60} = C_{30} \cdot e^{-d \cdot T}$$

where C_{60} = the concentration in mg. per L. existing at 60 minutes after injection
C_{30} = the concentration in mg. per L. existing at 30 minutes after jniection
e = the base of the natural logarithms
d = the decay constant
T = elapsed time

The equation can be solved for the decay constant to obtain:

$$d = \frac{\ln C_{30} - \ln C_{60}}{30}$$

where ln = natural logarithm of indicated quantity
30 = elapsed time in minutes
and other terms are as previously defined.

It is seen that the difference of the natural logarithms of 30- and 60-min

ute concentrations divided by elapsed time is equal to the decay constant.

Table 2 relates the computation carried out on 3 normal adult male subjects in whom values at 30 and 60 minutes were available. The decay, or disappearance, constant averaged .019. Other values in different subjects were available for computing the decay constant between 10 and 15 minutes, between 15 and 30 minutes, and between 35 and 60 minutes. The values are summarized in Table 3. While minor differences exist in the speed of the decay, the figures do not differ sufficiently to prove that the decay rate changed with changing concentration although this is probable. The values from 10 minutes on were averaged and an overall decay rate of .016 obtained.

The decay rate is related to percentage rate of disappearance but cannot be expressed as a simple multiple of the decay constant although in this particular case they are so related. The following computation shows this.

Assume the concentration existing at one minute is unity, and the time interval is unity, then the concentration existing at 2 minutes is:

$$C_2 = C_1 \cdot e^{-d}$$

where C_2 = the concentration in mg. per L. existing at 2 minutes after injection

C_1 = the concentration in mg. per L. existing at 1 minute after injection

and the other terms are as previously defined.

TABLE 2.—CALCULATION OF DECAY CONSTANT OF COOMASSIE BLUE FROM PLASMA CONCENTRATIONS RECORDED AT 30 AND 60 MINUTES IN 3 NORMAL ADULT MALE SUBJECTS

Case No.	*Conc. 30′ mg./L.*	*Conc. 60′ mg./L.*	*lnConc. 30′*	*lnConc. 60′*	*Difference (4–5)*	*Decay Constant Difference/T where T=30*
(1)	*(2)*	*(3)*	*(4)*	*(5)*	*(6)*	*(7)*
7	8.5	4.6	2.14	1.53	0.61	.020
8	18.0	10.1	2.89	2.31	0.58	.019
12	10.4	6.0	2.34	1.79	0.55	.018

Formula: $d = \frac{\ln C_{30} - \ln C_{60}}{T}$

TABLE 3.—CALCULATION OF DECAY CONSTANT OF COOMASSIE BLUE FROM PLASMA CONCENTRATIONS RECORDED AT THE INTERVALS: 10–15; 15–30; 30–60 AND 35–60 MINUTES IN THE INDICATED NUMBER OF NORMAL ADULT MALE SUBJECTS

N	*Time Interval minutes*	*d mean*
3	10–15	.0154
3	15–30	.0165
3	30–60	.0193
4	35–60	.0145
	Grand mean:	.0164

N = number of subjects in whom observations were made

The concentration existing at 2 minutes is the value obtained by raising the base of the natural logarithm to minus the decay rate. Entering a table of exponential functions, one ascertains the value of e to the minus .016 to be .984. This is the concentration at 2 minutes, under the conditions specified. Knowing the concentration at 1 minute and at 2 minutes, the percentage rate of disappearance can be computed:

$$\frac{C_1 - C_2}{C_1} \cdot 100 = 1.6\%$$

Biological half-life is properly computed using a formula with "t" unknown:

$$100 - 50 = e^{d \cdot T}$$

where: 100 = concentration of 100%
50 = concentration ½ of previous listed concentration or 50%
e = base of natural logarithm
T = time elapsed from C_{100} to C_{50}

Taking logarithms gives:

$$\ln 100 - \ln 50 = d \cdot T$$

Since ln 100 — ln 50 equals .693, this figure, divided by the decay constant, is equal to the biological half-life. This biological half-life differs from the physical half-life of radioactive isotopes in that the decline of dye concentration is manifested only *in vivo*, whereas isotope activity declines as a result of an intrinsic property independent of its presence in or out of an organism.

When the values for 15, 30 and 60 minutes are plotted on semi-logarithmic paper with the abscissa representing elapsed time and the ordinate representing concentrations, the line connecting the points can be extended back to zero time and a concentration value obtained representing what existed before decay had commenced and when mixing was complete. This is a fictitious, but useful value, analogous to the extrapolation of the linear disappearance curve used by Gibson and Evans[4]. The difference between Gibson's handling of his data and the method used here is that the rapid disappearance rate of the Coomassie Blue necessitated computation of the disappearance constant or the use of semi-log paper. By use of the theoretical mixing value at zero time, the plasma volume can be calculated. The results are shown in Table 4.

TABLE 4.—QUANTITY OF DYE INJECTED (I), CONCENTRATION OF DYE AT ZERO TIME; PLASMA VOLUMES, ABSOLUTE AND RELATIVE; BLOOD VOLUMES ABSOLUTE, AND RELATIVE

Case No. (1)	*I* mg. (2)	*Conc. "0" Time* mg. (3)	*Plasma Volume* ml. (4)	*Plasma Volume* ml./kg. (5)	*Blood Volume* ml. (6)	*Blood Volume* ml./kg. (7)
1	50	12.7	3937	49.90	6417	81.33
2	64	15.3	4183	48.53	7362	85.41
3	50	16.0	3125	49.21	5250	82.68
4	50	13.8	3623	49.29	6087	82.82
6	60	22.4	2679	40.71	4608	70.03
7	42.8	14.9	2873	34.61	4884	58.84
8	78.4	26.1	3004	39.22	5077	66.28
9	81.2	25.5	3184	40.56	4999	63.68
12	70.17	16.2	4332	63.71	7779	114.66
13	77.12	14.1	5470	65.91	8789	105.90
			Mean:	41.74		81.16
			Range:	34.61–65.91		58.84–114.66

Calculating total blood volume from plasma volume is inexact because of the necessity of using the packed cell volume for computation. This is explicit in any method using dye, since the first step in measuring dye concentration is separation of plasma from red cells by centrifugation. But the same dependence on plasma percentage derived from the hematocrit value exists even though the indicator concen-

tration be measured in blood as, for example, in the I_{131}-labeled albumin method. This is true because the albumin exists in the plasma and one is ascertaining the number of counts of radioactivity in this aliquot of plasma, implicitly assuming that the venous sample drawn contains the same fraction of plasma as does the blood in every other part of the body. The same limitation applies to methods which measure red cell mass if they are used to calculate plasma volume, employing the packed cell volume. No method exists which will measure blood volume in a single determination: plasma volume is measured or red cell mass is measured; from the hematocrit the other can be computed.

Two errors exist in the measurement of packed cell volume by centrifugation of a venous sample. One relates to "trapped plasma." That is, when centrifugation takes place some of the plasma lodges between the cells and produces an artificially high reading of the packed cell volume. Rapid centrifugation for 30 minutes is thought to minimize this error. Nevertheless, some investigators apply a correction factor of .96 to the hematocrit as recorded. The second error in the packed cell volume estimation is systematic rather than technical. It centers on the question: how representative of all the blood is the venous sample? It is known that arterial blood contains relatively fewer cells than venous blood. Hence a correction factor of .91 applied to the venous hematocrit has been suggested (Gregerson and Rawson[5]) and is used in this report.

From this latter value the blood volume can be calculated. Blood volumes so computed are shown in Table 4, last two columns.

Discussion. RELIABILITY OF THE DETERMINATION. The scatter of plasma volume values, reported in Table 4 is considerable. In general this is true of all plasma volume measurements, but in this instance the variability is greater than usually reported. In part, this can be attributed to the nonbasal state of the subjects but more importantly to the variable decay rate encountered with resultant difficulty in ascertaining a true zero time value, crucial to the determination. The Coomassie Blue method is not capable of greater precision until this question can be systematically answered as a result of further studies.

Summary. Coomassie Blue dye has been found safe and convenient to use without hazard in a study of 10 normal adult males. The behavior of the dye in the body, its extraction from dyed plasma and its ease of measurement in a spectrophotometer confirm the prior observations of Taylor and Shillingford. Referring to the second paragraph of this paper it can be seen that Coomassie Blue meets the requirements No. 1, 2, 3, 6 and 8 of the ideal indicator. Evidence is not sufficient to state that it meets requirements No. 4, 5 and 7.

The dye may have clinical usefulness in the determination of plasma volume if due attention is paid to its decay rate.

Coomassie Blue has proved satisfactory in recording arterial concentration curves in conjunction with an ear-piece transducer.

ACKNOWLEDGMENT: The author is indebted to Miss Maryann Henricksen, B.S. (A.S.C.P.), for the spectrophotometric determinations.

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SUMMARIO IN INTERLINGUA

Blau Coomassie, Usate in le Estimation del Volumine de Plasma: Reporto Preliminari

Comparation del Colorante con Altere Indicatores, con un Reporto del Valores Obtenite in Dece Normal Subjectos Adulte

Le colorante Blau Coomassie se ha provate innocente e de uso convenibile in un studio de 10 normal adultos mascule. Le comportamento del colorante in le corpore, su extraction ex plasma colorate, e le facilitate de mesurar lo in un spectrophotometro confirma le previe observationes de Taylor e Shillingford.

In le presente communication (vide paragrapho 2), octo criterios es enumerate que characterisarea le indicator ideal. Blau Coomassie satisface cinque de illos (1, 2, 3, 6, 8). Quanto al altere tres (4, 5, 7), le evidentia nunc disponibile es ancora inconclusive.

Le colorante Blau Coomassie promitte esser utile ab le puncto de vista clinic in le determination del volumine de plasma, providite que appropriate attention es prestate al cognoscite valor de su periodo de degeneration.

Blau Coomassie se ha provate satisfactori in le registration de curvas de concentration arterial, con le utilisation de un oxymetro auricular.

THE VALUE OF THE QUININE TEST FOR THE DIFFERENTIATION OF JAUNDICE

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Quinine, used extensively in the past for the treatment of malaria, was known to be inactivated in the liver (Grosser[7], 1906). It was found subsequently in experimental animals that this happened in the presence of a specific oxidase found exclusively in the liver cells (Kelsey *et al.*[10], 1943). The natural substrate of the enzyme is supposed to be the n-methyl-nicotinic acid amide (Knox[11], 1946), which has a pyridine nucleus. This nucleus is shared by quinine, which is also oxidized by this naturally occurring enzyme (Lang and Keuer[12], 1957, Baier and Lang[3], 1957). The existence of the enzyme in human liver has been demonstrated recently (Führ[6], 1952). The oxidized product of quinine is a carbostyril compound, which can be recognized by its physical characteristics, namely fluorescence and absorption in a certain wavelength.

Baier[2] (1958) searched for the quinine oxidase in the serum of patients with hepatocellular jaundice and reported it present in various quantities in hepatitis cases, and absent in cases of jaundice of other nature. He proposed the use of the determination of this oxidase, by a method described previously (Baier and Lang[3], 1957), as a diagnostic test for jaundice.

The simplicity of the test and the high proportion of positive results in hepatocellular damage seemed promising. Roman and Dulmanis[14] (1959) in a preliminary report to the Lancet presented data in accordance with Baier's findings, while Ballot and Terlingen[4] (1960) in another, also brief, report challenged the enzymatic nature of the test.

This is a report of a clinicolaboratory study of 120 icteric patients, 20 normal individuals and 20 patients with other diseases, in an effort to evaluate the quinine test and elucidate its nature.

Materials and Methods. The patients of the study were hospitalized either in our Department or in other Hospitals of Athens and Piraeus*. In every patient, in addition to the quinine test, the Jirgl flocculation (Jirgl[8], 1957, Daikos, Mattheou and Athanasiadou[5], 1959), the routine flocculation liver function tests, the bilirubin content of the serum, the alkaline phosphatase and in some cases the oxalacetic acid transaminase, were performed. A surgical or pathological

*We are indebted to the Directors and staff of the following Hospitals for their help in studying cases hospitalized in their Services: Department of Propedeutic Clinic of the Athens University Medical School, Hippokrateion Hospital (Medical and Surgical Departments), Evangelismos Hospital (3rd and 4th Med. Services), King's Paul Hospital (Piraeus), Veterans Administration Hospital, St. Helen's Hospital.

TABLE 1.—SUMMARY OF FINDINGS OF VARIOUS TEST

Diseases	*Number of cases*	*Quinine test. Normal values 0–6 U./ml.*			*Jirgl test*			*Cephalin flocculation*		
						Positive			*Positive*	
		Negative	*Positive*	*Values in units*	*Negative*		*degree flocul.*	*Negative*		*degree flocul.*
Hepatitis Infectious and homologous serum	50	—	50	10–66	45	5	+ to +++	2	48	++ to ++++
Toxic Hepatitis	3	—	3	35–85	2	1	+		3	++ to ++++
Cholangiolitis	3	2	1	14	2	1	+	—	3	++ to ++++
Liver Neoplasms	9	1	8	8–35	3	6	+ to +++	4	5	+ to +++
Liver cirrhosis	15	11	4	14–26	13	2	++	2	13	+ to +++
Cholangiitis (with cholelithiasis)	5	—	5	14–45	1	4	++ to +++	1	4	+ to +++
Obstructive Jaundice Chololithiasis, Neoplasms of pancreas, etc.	30	26	4	8–14	8	22	+ to ++++	23	7	+ to +++
Hemolytic jaundice	5	5	—		5	—		4	1	++
Various diseases	20	20	—		20	—		16	4	+ to ++
Normal individuals	20	20	—		20	—		19	1	+

confirmation of the diagnosis was pursued, wherever possible.

The quinine test was performed as follows (Baier[2], 1958): The optical density in 366 m/micra of a mixture of 1 ml. of the tested serum and 3 ml. of 0.066 M phosphate buffer of pH 7.2 was measured 1 minute and 10 minutes after the addition of 1 ml. of aqueous solution of chemically pure crystalline quinine hydrochloride 0.025%. An increase in optical density of 0.001 in 10 minutes is taken as an enzyme unit. A tube containing 0.9 ml. of serum and 4.1 ml. of buffer served as a control. The results are expressed in Units per 1 ml. of serum, normal values being considered 0 to 6 U. per ml.

In 30 quinine-positive (with the highest values observed; that is, from 25 to 85 U. per ml.) and in 10 negative sera we searched for the oxidized product of quinine, which should be present in the sera if the test is actually measuring quinine oxidase. The physical characteristics of quinine carbostyril are: a) that its maximum of absorption lies in the 352 m/micra wavelength, b) that it fluoresces intensely in a borate buffer of pH 10 (Knox[11], 1946).

FLUORESCENCE. One milliliter of the tested serum is placed in each of 2 test tubes containing 3 ml. of the phosphate buffer (pH 7.2) and then 1 ml. of a 0.025% aqueous solution of chemically pure quinine hydrochloride is added to both tubes. In the one test tube, named A, after 1 minute and in the other, named B, after 10 minutes, 5 ml. of a recently made 5% metaphosphate solution are added. The test tubes are left to stand in room temperature for a few minutes. Then the contents are filtered through regular filter paper. In the protein precipitate quinine is adsorbed while the oxidized product remains in the filtrate (Knox[11], 1946). The pH of the clear filtrate is corrected to 10 through the addition of borate buffer of pH 10.14. The fluorescence is examined in different wavelengths. Any eventual difference in fluorescence between the test tubes B

IN 120 CASES OF JAUNDICE AND 40 CONTROLS

Thymol turbidity normal values in McLagan U./1–4			*Zinc Sulfate flocculation Normal values in McLagan U.*/2–8			*Serum bilirubin mg/100 ml normal values*	*Alkaline Phosphatase. Normal values in Bodansky U.*/3–5		
	Positive			*Positive*				*Increased*	
Negative		*Units*	*Negative*		*Units*	*0.25–0.50*	*Normal*		*Units*
1	49	4,5–18	1	49	9–29	1,20–26	12	38	5,5–32
	3	8–13	—	3	11–16	8–32	—	3	7–14
1	2	4,5–15	1	2	10–12	2,5–8	—	3	8,5–20
4	5	5–25	4	5	8,5–44	0,8–22	1	8	10–75
3	12	4,5–13	5	10	10–30	0,8–12	3	12	7–42
1	4	6–9,5	1	4	10–40	0,9–11	1	4	14–35
24	6	4,5–9,5	24	6	10–32	0,8–25	1	29	7,5–60
5	—		5			0,9–2,6	5	—	
19	1	5	18	2	8,5–10	0,2–0,5	20	—	
20	—		20			0,2–0,5	20	—	

and A is recorded. At the same time the fluorescence of a blank containing all the above mentioned solutions, except the serum, is checked.

After the above mentioned deproteination of the samples A and B, the pH of the filtrate is brought to 7.2 through the addition of carbonate buffer of pH 10 (which is not found to interfere with the system, Knox[11], 1946). Then the difference in optical density between B and A is measured in a wavelength of 366 m/micra. The existence of the oxidized product should be manifested by a difference in optical density.

MULTIPLE CONTROLS according to the modification of Ballot and Terlingen[4] (1960) were set up in 10 negative and 30 positive sera, as follows: Three samples of each serum were tested in the following combinations. In each of 3 test tubes 1 ml. of serum and 3 ml. of phosphate buffer (pH 7.2) were placed. a) In the first test tube 1 ml. of aqueous quinine dilution (0.025%), b) in the second 1 ml. of distilled water, c) in the third 1 ml. of 0.025% quinine solution in phosphate buffer (pH 7.2) were added. The measurement of optical density was performed as in the quinine test described previously, 1 and 10 minutes after the addition of the last solution.

Results. As shown in Table 1, all cases of infectious and "toxic" hepatitis gave a positive quinine test, with a wide range of units (10 to 85 U. per ml. of serum). The test was also positive in one of 3 cholangiolitis cases, in 8 of 9 liver neoplasms, in 4 of 11 cirrhotics, in 5 of 5 cholangiitis (cholelithiasis with infection of the bile canals) and in 4 of 30 obstructive jaundice cases. All 5 cases of hemolytic jaundice gave a negative quinine test. The highest values were observed in the

hepatocellular icterus, being correlated with the severity of the case.

The Jirgl flocculation test came out slightly different than has been previously reported (Daikos, Mattheou and Athanasiadou[5], 1959). The majority of the hepatitis cases gave a negative test as expected: 45 of 50 infectious hepatitides, 2 of 3 cholangiolitides and 2 of 3 "toxic" hepatitides. Of the liver neoplasms (with obstruction) 6 gave positive and 3 negative tests. Of the obstructive jaundice cases 26 out of 35 (listed in the table as obstructive jaundice and cholangiitis) came out positive (74.25%). If we include the liver neoplasms, all of which had obstruction, 32 out of 44 obstructive jaundice cases (72.75%) gave a positive Jirgl flocculation test.

A comparison with the routine flocculation tests shows a good correlation of the quinine test with cephalin flocculation, thymol turbidity and zinc sulfate tests. Alkaline phosphatase was found slightly elevated in cases of hepatocellular jaundice and more elevated in obstructive jaundice.

LABORATORY EVALUATION OF THE QUININE TEST. The results of the tests designed to evaluate the quinine test are consistent with the view that the test does not measure any enzyme oxidizing quinine, but it is of a different and as yet unclear nature. Thus: 1) no difference in fluorescence was observed in the quinine-positive and negative sera between tubes B and A (*vide supra*). The observed fluorescence was not greater than the slight one given by the blank. 2) No difference in optical density was observed in quinine-positive and negative sera between tubes A and B. In a few sera there was a negative and not a positive difference, as expected. 3) No difference in optical density was observed in any of the tested sera in tube (c) that is, the tube that contained the quinine solution in phosphate buffer. On the other hand, in all 30 positive sera tested the same difference in optical density was observed in each case both in the tube (a) containing the aqueous solution of quinine and in the tube (b) containing the distilled water.

There is no doubt that the positive quinine test, which comes out positive in the same degree both in the presence of aqueous quinine solution and of distilled water, is not of enzymatic nature.

Discussion. The quinine test came out positive in all cases of hepatocellular damage. No case of infectious, or toxic, hepatitis gave a negative test. On the other hand, some cases of neoplasms, some of the liver cirrhoses and the infected obstructive jaundice, where we can presume a degree of hepatocellular damage, gave positive results. The highest values were observed in true and severe cases of hepatitis. On the contrary, the pure obstructive jaundice cases came out negative. The same was true for the hemolytic icterus cases.

The results of the quinine test correspond to a great extent with the Jirgl flocculation test, which came out slightly different than previously (Daikos, Mattheou and Athanasiadou[5], 1959). In the majority of the hepatocellular jaundice cases (47 out of 53) it was negative, no flocculation being observed, except in a few cases of hepatitis. In the majority of obstructive jaundice cases the Jirgl flocculation test came out positive (in 26 of 35, 74.25%). The hemolytic jaundice cases gave a negative test, as has been observed previously.

Of the two tests, the quinine test offers the advantage of simplicity and ease of performance. The Jirgl test is also simple, but not as simple as the quinine. The results of the quinine test were more clear-cut and the pro-

portion of positive in the hepatitis cases was 100%. It seems that it reflects more truly the hepatocellular damage. We think that it can be helpful in our armamentarium for icterus diagnosis. In combination with the Jirgl flocculation test, it offers good information in most cases.

We think that there remains little doubt that the quinine test does not reflect any enzymatic activity and does not show the presence of quinine oxidase in the serum. The presence of the oxidized quinine product, quinine carbostyril (found by previous investigators in liver slices of laboratory animals) has not been proven in our sera with strongly positive quinine test. No trace of fluorescence or absorption has been observed in any positive deproteinated sera after 10 minutes contact with aqueous quinine solution. The most convincing evidence, however, comes from the multiple control of the test as proposed by previous investigators (Ballot and Terlingen[4], 1960). The test was positive in all sera tested in the absence of quinine, with distilled water alone and phosphate buffer. This is strong evidence against the enzymatic nature of the test. It should be taken into consideration that the way we searched for the fluorescence entails an approximately fourfold dilution of the test tube contents, but this should not prevent, in the light of the findings of earlier workers (Knox[11], 1946), the detection of the oxidized product which fluoresces intensely even in minute amounts.

Our findings, therefore, are consistent with the view that the quinine test is a valuable diagnostic tool in jaundice cases, but it does not detect any quinine oxidase in the serum. It should perhaps reflect disturbances of protein balance in the serum of patients with hepatocellular jaundice.

Summary. The value of the quinine test for the diagnosis of liver damage has been investigated in 120 icteric patients, 20 normal individuals and 20 patients with other diseases. In all hepatitis cases the test came out positive, in contrast to obstructive jaundice cases where, in the majority, it came out negative. These findings are correlated with the previously described Jirgl flocculation test, which came out approximately similar to previous findings, that is, negative in hepatocellular jaundice and positive in obstructive jaundice. None of the tests seems specific. The quinine test does not prove the existence of any enzyme in the serum. Its value, nevertheless, remains considerable for the diagnosis of jaundice, since it is so simple in performance and so consistently positive in hepatitis cases.

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SUMMARIO IN INTERLINGUA

Le Valor del Test a Quinina in le Differentiation de Jalnessa

Le valor del test a quinina in le diagnose de lesiones hepatic esseva investigate in 120 patientes icteric, 20 subjectos normal, e 20 patientes con altere morbos. In omne le casos de hepatitis, le resultatos del test esseva positive, per contrasto con le casos de jalnessa obstructive ubi—in le majoritate del casos—le resultatos esseva negative. Iste constatationes es correlationate con le previemente describite test de flocculation de Jirgl. Le resultatos del test de Jirgl esseva approximativemente le mesmes como in previe series, i.e., illos esseva negative in casos de jalnessa hepatocellular e positive in jalnessa obstructive. Il pare que nulle de iste tests es specific. Le test a quinina non prova le existentia de ulle enzyma in le sero. Su valor remane nonobstante considerabile in le diagnose de jalnessas, gratias al facto que illo es si simple a executar e que su resultatos es si uniformemente positive in casos de hepatitis.

THE INFLUENCE OF AGE AND SEX ON HUMAN PLASMA AND RED CELL CHOLINESTERASE*

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The influence of sex on human plasma cholinesterase activity has been studied by many investigators. The reported findings, summarized by Augustinsson[3], were controversial. Augustinsson[3], himself, found the plasma cholinesterase activity of males to be significantly higher than that of females. Since his review, however, several other reports appeared, some of which (Dixon[11], Rider *et al.*[20]) upheld his findings, while others (Boulvin[8], Moore *et al.*[18], Pribilla[19], Sakaino[21]) claimed that there is no difference between the plasma cholinesterase activity of the two sexes. Considerably less information is available on the influence of age on the activity of human plasma cholinesterase. Lehman, Cook and Ryan[12] and Brody[9] reported that plasma cholinesterase activity was significantly lower in the newborn than in adults. Several workers found no difference between the plasma cholinesterase level of middle-aged and old individuals (Borders *et al.*[7], Boulvin[8], Limperos and Ranta[16]) while others reported a decrease of the activity of this enzyme in the aged (Barrows, Shock and Chow[4], Bertola and Castellani[5], Cresti and Barbato[10]).

In contrast to the findings reported on plasma cholinesterase, most available data indicate that neither age nor sex has a significant effect on human red cell cholinesterase activity (Augustinsson[3], Dixon[11], Limperos and Ranta[16]).

In view of the extensive clinical use of compounds (for example, ester-type local anesthetics and neuromuscular blocking agents) which are broken down by plasma cholinesterase, it seemed worth while to investigate the influence of age and sex on the activity of this enzyme. Besides acetylcholine,

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butyrylcholine and benzoylcholine generally employed for the determination of plasma cholinesterase activity, succinylcholine and procaine, two clinically significant compounds, were also utilized as substrates. In addition, the influence of age and sex on the hydrolysis of acetylcholine and acetyl-β-methylcholine by red cell cholinesterase was also observed. A preliminary report on this subject was published elsewhere (Shanor *et al.*[22]).

Material and Methods. The investigation was carried out on 50 healthy, young, human volunteers, whose ages ranged from 18 to 35 years, and on 50 surgical patients with no known pathology (for example, liver disease) that could have affected plasma cholinesterase activity. The ages of the second group ranged from 70 to 80 years. Both groups were evenly divided between the two sexes.

Ten milliliters of heparinized blood were removed from each subject at least 12 hours after the last meal. The blood was centrifuged within 15 minutes after its withdrawal. Plasma cholinesterase activity was determined by Ammon's[1] modification of Warburg's manometric method with acetylcholine, butyrylcholine, benzoylcholine and succinylcholine substrates, and with Kalow's[14] ultraviolet spectrophotometric method with procaine substrate. The substrate concentration of procaine was 5.0×10^{-5}M and that of the other substrates 2.2×10^{-2}M. The final plasma dilutions v/v were with butyrylcholine, 1:100; acetylcholine and benzoylcholine, 1:50; procaine, 1:10 and succinylcholine, 1:5.

Red cell cholinesterase activity was determined with Ammon's[1] manometric method using washed, hemolyzed red cells prepared according to the technique of Augustinsson[2] with acetylcholine and acetyl-β-methylcholine substrates. The substrate concentration of acetylcholine was 3.1×10^{-3}M and for acetyl-β-methylcholine 2.2×10^{-2}M. The final red cell dilution v/v was 1:330. Otherwise, the experimental conditions were the same as in the plasma cholinesterase studies.

The results were expressed as micromoles hydrolyzed by 1 ml. of plasma or red cells in 30 minutes. All determinations were made in duplicate at pH 7.4 and 37° C. Correction was made for nonenzymatic hydrolysis.

Results. The results are summarized in Tables 1 to 5. Table 1 indicates that female plasmas hydrolyzed the various substrates at a rate ranging from 64 to 74% of that of the male rate. These differences were statistically significant, with p values less than 0.01. In contrast to the findings with plasmas obtained from young adults, no significant difference was found in the plasma cholinesterase activity of aged males and females (see Table 2). Similarly, there was no difference between the plasma cholinesterase activity of aged and young females. The only exception was that procaine was hydrolyzed faster by young than by old females (see Table 3). Statistically significant differences were found, however, between the hydrolysis rates of the different substrates in plasmas obtained from young and aged males (see Table 4). According to Table 5, there is no statistically significant difference between the red cell cholinesterase activity of young males and females.

TABLE 1.—COMPARISON OF THE HYDROLYSIS OF VARIOUS SUBSTRATES IN THE PLASMAS OF HEALTHY YOUNG ADULTS OF BOTH SEXES

Substrates	*Males*	*Females*	*t-value*	*p-value*
Acetylcholine	122.8* ± 6.30†	83.1 ± 5.60	4.7	<0.01
Benzoylcholine	43.4 ± 2.60	28.1 ± 2.20	4.5	<0.01
Succinylcholine	3.0 ± 0.10	2.1 ± 0.10	5.0	<0.01
Butyrylcholine	259.3 ± 12.20	179.6 ± 13.70	4.7	<0.01
Procaine	0.658 ± 0.03	0.488 ± 0.02	4.6	<0.01

*Micromoles of substrate hydrolyzed by 1 ml. of plasma in 30 minutes.
†Standard error.

Discussion. The findings presented regarding the influence of sex and age on plasma and red cell cholinesterase activity are in agreement with those of Augustinsson[3] and Barrows, Shock and Chow[4], respectively. Their data were obtained with the use of one substrate for each enzyme. In the present investigation, however, besides the conventional substrates of plasma cholinesterase, two pharmacologically and clinically important ester-type drugs, procaine and succinylcholine, were also used to test the activity of this enzyme.

At present, no definite explanation is available for the differences observed between the plasma cholinesterase activity of young males and females or young and old males. The finding of a suitable explanation is made more difficult by the lack of any known definite physiological role of plasma cholinesterase. By using selective inhibitors, it is possible to inhibit plasma

TABLE 2.—COMPARISON OF THE HYDROLYSIS OF VARIOUS SUBSTRATES IN THE PLASMAS OF AGED MALES AND FEMALES

Substrates	*Males*	*Females*	*t-value*	*p-value*
Acetylcholine	93.9* ± 7.00†	88.5 ± 6.20	0.50	>0.5
Succinylcholine	2.2 ± 0.10	2.3 ± 0.10	0.05	>0.5
Procaine	0.398 ± 0.03	0.392 ± 0.03	0.16	>0.5

*Micromoles of substrate hydrolyzed by 1 ml. of plasma in 30 minutes.
†Standard error.

TABLE 3.—COMPARISON OF THE HYDROLYSIS OF VARIOUS SUBSTRATES IN THE PLASMAS OF YOUNG AND AGED FEMALES

Substrates	*Young*	*Aged*	*t-value*	*p-value*
Acetylcholine	83.1* ± 5.60†	88.5 ± 6.20	0.65	>0.5
Succinylcholine	2.1 ± 0.10	2.3 ± 0.14	1.16	>0.2
Procaine	0.49 ± 0.02	0.39 ± 0.03	2.39	<0.01

*Micromoles of substrate hydrolyzed by 1 ml. of plasma in 30 minutes.
†Standard error.

TABLE 4.—COMPARISON OF THE HYDROLYSIS OF VARIOUS SUBSTRATES IN THE PLASMAS OF YOUNG AND AGED MALES

Substrates	*Young*	*Aged*	*t-value*	*p-value*
Acetylcholine	122.8* ± 6.30†	93.9 ± 7.00	3.1	<0.01
Succinylcholine	3.0 ± 0.10	2.2 ± 0.13	4.9	<0.01
Procaine	0.66 ± 0.03	0.4 ± 0.03	6.1	<0.01

*Micromoles of substrate hydrolyzed by 1 ml. of plasma in 30 minutes.
†Standard error.

TABLE 5.—COMPARISON OF THE HYDROLYSIS OF VARIOUS SUBSTRATES IN THE HEMOLYZED RED CELLS OF HEALTHY YOUNG ADULTS OF BOTH SEXES

Substrates	*Males*	*Females*	*t-value*	*p-value*
Acetylcholine	270.5* ± 9.4†	265.2 ± 11.0	0.38	>0.5
Acetyl-β-methylcholine	86.0 ± 7.6	84.5 ± 4.2	0.62	>0.5

*Micromoles of substrates hydrolyzed by 1 ml. of hemolyzed red cells in 30 minutes.
†Standard error.

cholinesterase completely, both in man (Mazura and Bodansky[17]) and in animals (Bodansky[6]), without any apparent change in any physiological function.

Since the higher plasma cholinesterase activity of males decreases in old age, and its value approaches that of young and old females, it is conceivable that there is some correlation between the male sex hormone and the level of plasma cholinesterase.

In contrast to its unknown physiological significance, plasma cholinesterase has considerable pharmacological importance. As already mentioned, the ester-type local anesthetic agents as well as succinylcholine, a widely used muscle relaxant, are both broken down by plasma cholinesterase. Low plasma cholinesterase activity, therefore, makes patients more susceptible and high cholinesterase activity more resistant to the pharmacological and toxic effects of these compounds (Foldes *et al.*[12,13]). This may partly explain the clinical observation that young adult males are less sensitive to the toxic effects of local anesthetic agents and succinylcholine than females or aged males.

The finding that, in contrast to other substrates, procaine was hydrolyzed more slowly by plasmas obtained from aged females than by plasmas of young females, raises the possibility that the enzymes responsible for the hydrolysis of procaine on one hand, and the various choline esters on the other, are not identical.

Summary. 1. A statistically significant difference was observed between the plasma cholinesterase activity of young, healthy males and females. The activity of female plasmas with the substrates used was 64 to 74% of the male rate.

2. No sex variation was found in the plasma cholinesterase activity of old males and females.

3. The plasma cholinesterase activity of young males was about 24% higher than that of old males.

4. Except with procaine substrate, no significant difference was found between the cholinesterase activity of young and old female plasmas.

5. No statistically significant age or sex variation was found in red cell cholinesterase activity.

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SUMMARIO IN INTERLINGUA

Le Influentia de Etate e Sexo Super le Cholinesterase de Plasma e Erythrocytos Human

1. Un statisticamente significative differentia esseva observate in le activitate de cholinesterase del plasma inter normal juvene adultos mascule e normal juvene adultos feminin. Le activitate del plasmas feminin, con le substratos usate, esseva 64 a 74% del correspondente valores mascule.

2. Nulle variation secundo le sexos esseva trovate in le activitate de cholinesterase de plasma in subjectos de etates avantiate.

3. Le activitate de cholinesterase de plasma in juvene masculos esseva circa 24% plus alte que illo de masculos de etate avantiate.

4. Nulle significative differentia inter le activitate de cholinesterase de plasma in juvene femininas e illo in femininas de etate avantiate esseva trovate, excepte con le uso de un substrato a procaina.

5. Nulle statisticamente significative variation secundo etate o sexo esseva trovate in le activitate de cholinesterase in erythrocytos.

PROGRESS
OF
MEDICAL SCIENCE

PATHOLOGY AND BACTERIOLOGY

UNDER THE CHARGE OF

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AMEBIASIS

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THE term "Amebiasis" was introduced by Musgrave and Clegg[73] in 1906 and is used clinically to denote all those conditions which are produced in the human host by infection with *Entameba histolytica*. *Entameba histolytica* is a protozoon belonging to the group Rhizopoda. The parasites belonging to this group are characterized by their type of movement[1,4-7,9-12,16,19,26-28,29,31,32,37,42-45,48,65,81,82,89,96,101,106]. The members of this class move by means of extensions of the ectoplasm, or pseudopodia. It contains both parasitic and free-living species. A few of the former parasitize human beings. They pass their life cycle in a single host but for the continuance of their species, a transference of host is required. They are transmitted from man to man through the encysted stage.

Amebiasis is a worldwide problem, but it is more common in the tropics and the subtropics. The facility of modern transportation throughout the globe has aided the transmission and carriage of the disease from one part where it is commoner to other parts where it is less common.

The order Amebidae consist of the Genera Entameba, Endolimax, Iodameba and Dientameba. These are distinguished microscopically by their nuclear pattern and the nature of the cysts they produce. They consist morphologically of a single cell-like unit containing a nucleus or nuclei embedded in a matrix called the cytoplasm. The cytoplasm is usually coarsely or finely granular in appearance in the living organism and is divided typically into an outer portion called the ectoplasm and an inner portion, the endoplasm, that usually comprises most of the cell. The cytoplasm or the outer layer of the cytoplasm is concerned with the functions of motility, procurement and ingestion of food, ex-

cretion, respiration and protection. The organs of locomotion or pseudopodia consist of the prolongation of the ectoplasm into which the endoplasm flows. This typifies the movement known commonly as 'ameboid motion.' It is like a slug moving across the field of vision. The ectoplasm is also believed to be the site of contractile vacuoles. Contractile vacuoles have the functions of regulating the osmotic pressure and the digestion and elimination of food substances, but such vacuoles usually do not occur in any of the parasitic species.

The ectoplasm serves to some extent also as a protective covering for the cell. In numerous species, as a normal part of the life cycle, or when the 'vegetative or trophozoite' stage of existence becomes disadvantageous or impossible because of local conditions the ectoplasm thickens, becomes much more resistant and forms a protective covering on the cysts which develop under such conditions.

The endoplasm fulfills the function of nutrition and, because the nucleus is contained within it, is intimately concerned with reproduction. It also contains food vacuoles, ingested materials, and chromidia and chromidial bodies in some species of the protozoa. The endoplasm usually appears more coarsely granular than the ectoplasm when the two can be distinguished.

The nucleus is the most important body present in the endoplasm because of its functions in maintaining life and reproduction. It is of great importance for microscopical identification of the organism. The nucleus of the genus Entameba or Endameba is characterized by a layer of granules inside the nuclear membrane. The karyosome is compact and situated centrally in *Entameba histolytica* and eccentrically in *Entameba coli.* The Endolimax nucleus has no granules on the nuclear membrane. Its karyosome is a large irregular mass, either situated centrally or in contact with the nuclear membrane; it may be connected with another smaller mass by a delicate filament. The Iodameba nucleus has no granules on the nuclear membrane and its karyosome is a large circular mass surrounded by a ring of refractile globules. In Dientameba there are two nuclei. In each nucleus the karyosome consists of a mass of chromatin granules (usually six) embedded in a matrix of plastin.

Reproduction is by fission of the cell and this is always preceded by fission of the nucleus. When reproduction occurs in the cyst, the nucleus divides into 4 to 8 daughter nuclei that retain the characteristic structure of the trophozoite stage. During maturation of the cyst, a glycogen mass and chromatoid bodies appear. They are usually absent in the fully developed stage.

The genus Entameba or Endameba affecting man comprises the species histolytica which is pathogenic, the species coli or intestinal ameba and the gingivalis or mouth ameba, both of which are harmless in man. It was Leidy[64] who gave, in 1879, the generic name Endamoeba; the type species was *Endameba blattae,* parasitic to the cockroaches. Casagrandi and Barbagotto[16], in 1895, introduced the term Entameba for the generic name of the intestinal ameba of man. Dobell[35] pointed out that there were sufficient morphological differences to justify the two generic names and he named the coli as *Entameba coli* and the parasites of the cockroach as *Endomeba blattae.* The term Entameba is commonly retained by British authors while their American colleagues prefer to use Endameba for the intestinal ameba of human beings.

Stillwell has written a most interest-

ing review[94] of the history of amebiasis to which the author is greatly indebted. In it he notes that the discovery of amebae in man was first published in 1894 in Moscow by Gros[48], who described at that time the nature of '*Ameba gengivalis.*' These parasites were the saprophytic amebae later to be named *E. gingivalis.* He described them as vesicles found in the tartar of the teeth. These microscopic bodies moved slowly and adopted various shapes by means of ameboid extension and contraction. 'Globules' that moved were seen inside the parasites. He included in the article five drawings that illustrated various shapes assumed by the amebae.

Lambl[63] published, in 1859, a 58-page article dealing with objects found in the stool, and, in 1860, Loschner and Lambl published in Prague a monograph about the pathological lesions in disease in children. Two pages in this monograph, written by Lambl, were concerned with 'Amoeben.' Because of these publications, Lambl usually receives credit for being the first to see amebas in the feces of man but it is probable that he did not do so.

Stilwell[94] notes that Leuckart disputed, in 1863, that the organisms pictured by Lambl were amebae, since they had a likeness to organisms found in fowls that were eventually classed as Trichomonads.

Dobell[33,34] has maintained that Timothy Richard Lewis[65], an assistant surgeon in Her Majesty's British Forces, who was attached to the Sanitary Commissioner with the Government of India was the first man to see amebas in human feces. In the 1869 Annual Report of the Sanitary Commissioner with the Government of India, dated 1870, there was a report on cholera. Lewis' portion of the report consisted of an appendix in which he described and illustrated a number of objects found in the stool of patients suffering from cholera. Stilwell[94] has selected the following pertinent quotation, "Intermixed with the corpuscles already described are others to which I wish to allude with the greatest caution. Frequently a globule has been observed for some time and finally disposed as being merely an oil one, when suddenly it is seen to protrude a portion of its substance, retract it, and while so doing, another protrusion becomes visible at some other portion of the little mass and then, perhaps it will shift its position, exactly after the manner of an ameba." Lewis drew no conclusions concerning the significance of the presence of these objects. The sort of movement described may be that of *Entameba coli* and apparently is not the true locomotion that is typical of *Entameba histolytica.*

Stilwell[94] records that the next significant publication came from Russia in 1875. The article "Massive Formation of Amebas in the Large Intestine" was written by F. Lösch. The case was that of a 24-year-old Russian farmer observed in St. Petersburg from November 3, 1873, until his death on April 12, 1874. The patient had bloody stools containing much mucus, and the course of relapsing amebic dysentery.

Lösch observed the usual fecal elements and also saw motile amebae that contained red blood cells. Stilwell quotes him in translation as follows: "one can observe a large number of cellular structures which have a round, oval, pear-shaped or irregular form and which are in a state of almost continuous motion in which they change their form as well as their location. The movements were so fast and so peculiar that one was convinced, even on superficial examination, that one was not dealing with cellular elements which had either pathological or physiological amoeboid movement but with animal

organisms." He further stated "The nucleus is usually very pale, finely outlined and almost completely surrounded by the granular protoplasm. . . . It is especially difficult to convince oneself of their presence (nuclei) in those individuals that are resting and that contain a large number of granules.'" He named the organisms *Amoeba coli.*

In this same paper, Lösch described an experimental study of amebiasis. He injected fresh stools containing amebas into 4 dogs on 3 successive days both by anus and by mouth. Signs of dysentery appeared in one dog beginning 8 days after the final injection. Amebae like those from the patient's feces were recovered from the dog's stool. Eighteen days after the last injection, necropsy examination showed that the mucosa of the rectum was acutely inflamed and ulcerated, and amebae were present in the base of the ulcers and in the intestinal mucus. However, Lösch was not certain of the pathogenicity of the organisms. He speculated whether the illness could be entirely due to the amebae or if it might result from some other cause while the amebae were secondary invaders of the colon that only maintained the illness. In his view, comparison of the illness in the patient with the beginning of the disease in the dogs after injection of the stools, indicated great differences, and he conjectured that one must conclude that the patient "first took sick with dysentery, that the amebae gained access to the colon at a later date, multiplied and then maintained the inflammation."

At this time, Kartulis[56] began to contribute to the problem. He worked in Alexandria and in Egypt generally. In 1885, he reported "giant amebas" which he encountered in 6 cases, but they were not amebas, and what he may have seen is not known. Later he studied 150 patients in Egypt who had dysentery and found amebae in almost every patient. These observations were published in 1886. He also described the parasites in sections of intestinal ulcers and thought that they were the cause of dysentery in his patients. He also tried to culture the organisms and to inoculate them in animals to cause experimental dysentery but the attempt was unsuccessful.

Jaroslav Hlava[94], a professor of Pathologic Anatomy in Prague, published a 5-page article in a Czech medical journal in 1887. It was written in Czech and the title was "O Uplavici. Predbezne Sdeleni," which means "On Diarrhea. Preliminary Communication." He described amebas that he had found in 60 cases of dysentery and published illustrations. He also reported success in causing experimental amebiasis in cats by rectal inoculation of stools from dysentery patients.

At this time, a publication by Kartulis gave rise to a most fantastic episode in modern medical bibliography. In 1887, Kartulis published an abstract of Hlava's article. But by some curious mix-up the name of Hlava appeared nowhere in the abstract; instead the title of Hlava's work appeared in the place of the author's name. Thus, a new contributor bearing the name of O Uplavici (or, on diarrhea) appeared in the chronicle of amebiasis. Illustrations of the title page of both Hlava's article and Kartulis' abstract are given by Stillwell[94]. Kartulis made reference to an author of this coined name. The translated[94] passage of his work reads: 'Uplavici now makes the interesting statement that the European dysentery as well, is caused by amebas' . . . 'up to this time, Uplavici considered dysentery to be a mycotic disease.' Dr. Uplavici appeared in the bibliographies of amebic dysentery for many years. Hlava's paper was difficult to obtain

but in 1924 Dobell[35] succeeded in obtaining a typewritten copy of it and he laid the ghost of the spurious Dr. Uplavici to rest with a mock obituary.

About February 15, 1890, Osler[76] first saw in consultation a 29-year-old physician who had had chronic dysentery while in Panama. Two large hepatic abscesses were opened on March 22, 1890. Osler himself examined the pus and found many amebae. He made a number of drawings of what were undoubtedly *Entameba histolytica*. The data were published several weeks later. Osler's associates Councilman and Lafleur[24] published in 1891 a monograph on amebic dysentery. Stilwell writes[94]: "Their study was of great clinical importance because it crystallised for the first time the concept of amebiasis as an entity associated with definite pathologic lesions produced by amebas. Their representations of *E. histolytica* approached the modern ideas concerning the morphologic characteristics of the organism. They reemphasized the importance of ingested erythrocytes and called attention to one of the characteristic pathologic intestinal lesions, the so-called flask ulcer."

In 1893, Quincke and Roos[80] demonstrated in humans that more than one species of ameba might be present in the gut. They showed experimentally that one species of ameba produced dysentery but that the other species was harmless. They also discovered and illustrated amebic cysts.

During the next few years, a number of investigators published reports of their findings which confirmed and extended the observations mentioned above[2,3,8,13,19,20,29,30,33,38,47,48,52,53,55,66,68,69,79,81,82,86,96,97,99,102,106]. In 1903, Schaudinn[87] published an article on the propagation of various rhizopods in which he mistakenly described propagation by budding of sporulation as part of the life cycle. Other investigators supported Schaudinn's views and consequently there was confusion concerning the propagation of the organism for almost a decade.

Walker[96] published, in 1911, work done in the Philippine Islands and showed convincingly that *Entameba histolytica* and *Entameba coli* could be recognized by their different morphological characteristics although their general development was similar. The cysts of *Entameba histolytica* were noted to contain four nuclei, while those of *Entameba coli* contained eight. Contrary to the work of Schaudinn, Walker demonstrated that the multiplication of the amebae was not by budding but rather by repeated division of an original single nucleus.

Again, in 1913 Walker with Sellards[97] proved with the aid of volunteers that amebiasis could be produced by ingesting the cysts of *Entameba histolytica*. In 1924-1925 Boeck and Drbohlov[6] developed a special medium for the cultivation of amebae and in 1927 Craig[25] established a specific complement fixation test in the serum for the diagnosis of amebic infections. The final biological details of the life cycle of *Entameba histolytica* and *Entameba coli* were clarified a few years subsequently.

In his detailed account, Chatterjee[19], writing from India, notes that amebiasis is a world wide problem, but is more common in the tropical and subtropical countries than in the temperate zones. There is a wide variation of incidence of infection with *Entameba histolytica* in different countries[1,4,9,12,15,17,21,27,29,33,36,38,39,41,43,47,49,57,70,79,86,102]. Some authors use the term 'entamebiasis' in preference to amebiasis, because of all the intestinal amebae of man, the only species that is pathogenic falls under Entameba.

Some of the incidence figures that have been given are: in the United

States the incidence varies between 5 and 10%; in European countries, 8 and 10%; in Asiatic countries, 20 and 30%; in China, 30%; in Calcutta, 8 and 20%, and in Madras, 22%. The statistical records obtained by various authorities differ greatly. Moreover, in making surveys, the methods employed for the demonstration of the parasite by various observers have often been different so that the data must be evaluated according to the methods that were used.

In the Western hemisphere amebiasis has been found from Saskatchewan, Canada, 52° N. latitude), and Anchorage, Alaska (61° N. latitude) to the Strait of Magellan (52° S. latitude). The incidence of infection has been given as up to 85% in Mexico, 53% in Honduras and Colombia, 47 to 80% in Panama, and 44% in Haiti. In European countries Dobell[33] reported 7 to 10%, Wenyon[102] 11% and Boeck and Stiles[7] 15%. In a critical study of the distribution of amebiasis in the Western Hemisphere, Faust[42,43] has called attention to the fact that most data regarding the incidence of infection are much higher than the 10% postulated by Craig in 1926.

Age has a marked influence on the incidence of infection. Children below the age of 5 years are much less often infected than older children and adults. The greatest incidence of infection occurs between 26 and 30 years of age and a marked decline occurs after 30 years of age[19,29,31,70,86]. Males are more frequently infected than the females. *Entameba histolytica* is more frequent and more severe in its manifestations in the warm regions than the cold ones. Race is of little importance in the incidence of the parasite, as all races are susceptible to infection, but it is a frequent observation that the white race in the tropics often develops more severe symptoms than the native races. Social conditions have much to do with the incidence of infection in a given locality; it is an invariable observation that the poorer class shows a much higher incidence than the richer class.

Transmission of *Entameba histolytica* from man to man is effected through its encysted stage and infection occurs through the ingestion of these cysts. Fecal contamination of drinking water, vegetables, and food is of prime importance. Individuals suffering from active amebic diarrhea and dysentery pass trophic forms in their stool, which do not survive for more than a couple of hours and so are an unlikely source of infection. Moreover, ingested trophozoites are likely to be destroyed by digestion in the gastrointestinal tract. Those without any symptoms of the disease and others who are just convalescing may pass cysts in their stools and they are the individuals responsible for transmitting the infection from man to man. Food that has been handled by individuals who are cyst passers appears to be a very common source of infection. These persons suffer from only a mild attack of amebiasis and the parasites live in their gut in a state of equilibrium with the host's tissues. The lesions caused by the parasites in such persons do not produce any sharply defined symptoms but they do cause general weakness, digestive disturbances, abdominal pain and alternate constipation and diarrhea. Two types of carriers have been distinguished, namely the "contact" and the "convalescent."

Another important ally in the transmission of the disease is the house-fly. Flies convey the infection both directly and indirectly. The insects, while passing from feces to unprotected foodstuffs, carry the cysts mechanically. Moreover, the cysts of *Entameba histolytica* can pass unharmed through the alimentary canal of flies and, as

these insects defecate on the food material which they ingest, they may introduce living cysts into the food. The cysts of *Entameba histolytica* have also been found in cockroaches and they also may serve as a source of infection. Human excrement is often used as a fertilizer for growing vegetables. The eating of uncooked vegetables and unwashed fruits that may have been contaminated may cause infection. Occasionally, a water supply has been contaminated with infected feces and this has given rise to epidemics. In these instances, as chlorination does not kill the amebic cysts, the water should be boiled to kill them.

Man is the commonest reservoir and source of infection. In China, dogs are said to be a possible source of infection, and Boyd[9] and Ware[99] have reported the occurrence of natural infection with *Entameba histolytica* in dogs in India. Disease in monkeys has also been regarded as a source of infection in man. It has been shown that rats, although not an important factor in the spread of the disease in man, may be regarded as a reservoir of *Entameba histolytica*[2,20,66].

In man the incubation period is generally 4 to 5 days but it may vary a great deal. In their experimental work on human volunteers referred to earlier, Walker and Sellards[97] fed 20 men with cysts of *Entameba histolytica*. Eighteen of these men acquired the parasite and amebae were found in their stools from within 1 to 44 days, the average period being 9 days. Four of the 18 developed symptoms of amebic dysentery after intervals varying from 20 to 95 days from the time of ingestion of the cysts. Experimental studies with cats have shown that when cysts are given orally the incubation period is about 2 weeks, but if they are injected rectally it is only about 2 days.

The pathogenicity of *Entameba histolytica* depends not only on the organisms, but also on its environment in the host. There is a variable antagonism between the host and the parasite. Many secondary etiological factors including age, sex, diet, hygiene, climate and concomitant bacterial infections have been suspected or proven to affect the incidence or development of amebic disease. It is believed by many protozoologists that *Entameba histolytica* does not become pathogenic and cause lesions unless there is also a suitable or symbiotic bacterial infection. Streptococcus fecalis is normally present in the bowel and it is the bacterium that is incriminated. Similarly, an associated dysentery and paradysentery bacillary infection has been held to excite the clinical manifestations of amebic dysentery and render it refractory to specific antiamebic treatment. Consequently, it has been suggested that in such cases a successful control of secondary infection in the bowel will help the specific amebicidal drug to be more effective.

The clinical manifestations or signs and symptoms will vary according to the organs involved and according to the virulence of the infection. The term "amebic dysentery" used first by Councilman and Lafleur[24], in 1891, signifies a condition in which there is a dysentery with a stool containing blood and mucus and in which the infection is confined to the colon. Amebic dysentery is not synonymous with "amebiasis" which indicates the conditions caused by infection anywhere. Similarly, amebic dysentery is not the same as "intestinal amebiasis" since the latter may exist without dysentery[60,61].

The mature quadrinucleate cysts are the infective forms of the parasite. When swallowed by a susceptible person, they pass unaltered through the stomach, but are digested by the action of trypsin in the intestine causing ex-

cystation. Excystation may occur in the lower part of the small intestine but usually takes place when the cysts reach the cecum where the parasitic body comes out through a defect in the wall of the cyst. From each cyst there comes an ameba with four nuclei that undergoes binary fission and yields eight amebulas or metacystic trophozoites. The young amebae (trophozoites) are actively motile. If excystation has occurred in the small intestine they survive to reach the colon where they develop into mature trophozoites. Primary invasion of *Entameba histolytica* always occurs through the mucus membrane of the intestine where the ameba comes in contact with the columnar epithelium or through the crypts of Lieberkühn. The trophozoites make their way partly by direct mechanical penetration by virtue of their ameboid movement and partly by secretion of a powerful proteolytic enzyme called cytolysin which causes a mucosal erosion by dissolving the intestinal epithelial cells. The ameba can then invade the deeper layers of the wall of the colon by digestion of the tissue cells till they reach the muscularis mucosae. This muscle layer offers some resistance to lysis but they soon break through it and spread into the submucous tissues. The loose areolar tissue of the submucosa containing blood vessels and lymphatics presents little resistance to the migration of the parasites. The amebae multiply rapidly and form colonies as they invade. They destroy the tissues in their vicinity and utilize the lysed material as their food. Capillary blood vessels may be eroded leading to extravasation of blood in the necrotic tissue. The amebae then migrate in various directions, dissolving all tissues around them until a considerable area of the submucosa is destroyed, undermining the overlying mucous membrane, enlarging the mucosal defect and forming an abscess and ulcer. The amebae, discharged at first into the lumen, colonize and develop secondary lesions in other parts of the large gut. The process may lead to a relatively localized lesion or may disseminate and cause multiple and widespread lesions. In long-standing cases in which the whole length of the colon is involved, the oldest and most advanced lesion will be found in the cecum.

The lesions of amebiasis may be considered as those of primary or intestinal amebiasis causing diarrhea and dysentery, and those of metastatic amebiasis or secondary amebic infection. Metastatic lesions take origin from primary lesions of the colon and, while the patient may or may not display clinical evidence of intestinal infection, nevertheless, chronic latent ulcers can be found especially in the cecum and the ascending colon. The amebae that invade the bowel may enter into the radicles of the portal vein and be carried to the liver in which they may be arrested. Some may pass through the liver and enter the capillaries of the lungs. Again, they may be arrested or some may enter the systemic circulation. Localization and the production of lesions in the brain is seen, but with the exception of rare cases, the organisms seem unable to localize directly in other organs and tissues[9,13,18,21,23,40,46,49,53,58,59,71,74,75,76,77,78,81,82,84,85,88,90,91,92,95,100,104,105,107,108].

In a series of 186 cases of amebiasis, Clark[22] found that 113 cases showed generalized lesions scattered throughout the large gut, 63 cases showed only localized lesions, and 10 cases showed scars without any ulcers. In this series, Clark observed lesions of the appendix in one third. Two hundred and ninety cases of fatal amebiasis were filed at the Armed Forces Institute of Pathology from 1862 to

1953[59]. Of these, amebae were demonstrated during life or after death in 148 cases. In this series the principal involvement was as follows:

Location	*No. of Cases*	*Percentage*
Entire colon (multiple)	60	40.5
Cecum and terminal ileum	7	4.7
Cecum alone	21	14.2
Appendix	0	0
Cecum, ascending colon	14	9.5
Ascending colon	6	4.1
Transverse and descending colon	4	2.7
Rectosigmoid	11	7.4
Intestinal lesions absent:		
Liver abscess only	12	8.1
Liver abscess and lung involvement	3	2.0
Liver and brain abscess	2	1.4
Liver abscess bursting into pericardium	1	0.7

Chronic amebiasis with its sequelae and latent amebiasis are conditions that occur in patients who have had acute amebic dysentery, but they may also be observed in the absence of any previous history of diarrheal or dysenteric illness. The gastrointestinal disturbance is a diarrhea comprising frequent attacks of liquid stools and alternating with constipation. Palpation of the abdomen in chronic intestinal amebiasis may reveal either a thickening of certain portions or of the whole of the colon. Some patients may have a palpable mass or ameboma composed of inflamed tissue. The palpable lesions are usually near the umbilicus in the epigastric region (middle or transverse colon), or in the right or left iliac fossa. Such masses result from the destructive activity of the parasite together with the regenerative reaction of the host and may cause confusion as to the nature of the disease from which the patient suffers. Moreover, tenderness over the areas mentioned above has a location like that of gastroduodenal disease, chronic appendicitis or chronic cholecystitis and, consequently, amebiasis may be mistaken for them. Manson-Bahr[69] considered that a tender point located over the center of the sigmoid colon opposite the left anterior superior iliac spine is an "amebic sign post" because the sign is not found in other forms of colitis.

The sequelae of chronic intestinal amebiasis include neurasthenia that may lead to chronic invalidism; peritoneal irritation with the formation of adhesions; stenosis of the bowel due to healing of the ulcers by fibrous tissue; and spasm of the sigmoid colon. Rare sequelae include fibrosis of much of the colonic mucosa with the development of a sprue-like syndrome; and carcinoma of the colon ascribed to the chronic irritation caused by the presence of *Entameba histolytica* in the intestinal tissues or to the development of inflammatory "pseudopolyps" which may, in time, become malignant[83].

Latent amebiasis occurs with the development of a symbiosis between the parasite and the host and with the development of a partial resistance. The intestinal lesions may be small. The individual has *Entameba histolytica* in the colon and excretes amebic cysts in the formed stool. Such cases can act as carriers and infect a community.

The most important metastatic infection is hepatic amebiasis. It is manifested as (1) hepatitis and (2) liver abscess[18,21,23,46,58,59,74,75,76,77,78,84,85,88,90,91,92,93,100,104,105,107,108]. The trophozoites of *Entameba histolytica* enter small venules in the immediate vicinity of intestinal lesions, usually in the cecum and the ascending colon and pass in the portal venous circulation to the liver. The sinusoids of the liver arrest the parasites. Many of the parasites die without initiating hepatic lesions. However, if the invasion is mas-

sive or the host resistance is inadequate, some of the parasites will survive in the liver, they may then multiply in large numbers. If showers of amebae are frequently released into the portal circulation, a diffuse or widely disseminated infection of the liver that is amebic hepatitis develops. This condition does not cause extensive focal liver necrosis, but there are hepatomegaly, hepatic tenderness and fever. Subsequently, most of the amebae die. The remainder may multiply rapidly to form small local accumulations of amebae.Vascular channels become obstructed by pressure and thrombosis causing ischemic necrosis of liver cells. Lysis is promoted by the trophozoites. The lesion extends peripherally. It is a focus of necrosis that represents the initiation of a "liver abscess." Small abscesses may become confluent with adjacent foci or be part of an area of massive necrosis so that single or multiple large abscesses can develop.

Direct extension of amebae through the hepatic flexure of the colon into the liver is possible, but the organisms cannot reach the liver or disseminate in it along the bile ducts since bile is toxic to them.

Secondary bacterial infection of amebic abscesses of the liver may be seen in long-standing lesions. The usual routes of hepatic infection may act as the portals of entry. An unusual portal may occur when an abscess has extended to involve the lung. *Streptococcus hemolyticus, Streptococcus viridans, Staphylococcus aureus, Escherichia coli* and *Salmonella enteritidis* are among the bacterial organisms that may be recovered.

Metastatic amebiasis involving the lung is common[96]. Infection of the tissue of the right lung generally results from direct extension of a liver abscess through the diaphragm. Hematogenous infection of the lungs by parasites that filter through the liver from the bowel also occurs. The pathogenesis of a lung abscess is similar to that of a liver abscess. The incidence of amebic abscess of the lung, as a complication of liver abscess, has been found to be about 8 to 10% of cases[47,56,69]; it is rare in the absence of liver abscess. On occasion a liver abscess may drain through the bronchial tree.

Cerebral amebiasis is a rare variety of metastatic amebiasis[50]. Amebic brain abscess was first reported by Kartulis in 1904[94], who observed this complication in 3% of his cases of amebic dysentery. Other cases are mainly reported from Egypt. Most cases have been reported in patients with amebic liver or lung abscesses. Only a few cases have been reported as direct complications of intestinal amebiasis. An amebic brain abscess is usually solitary, of small size, and situated in one of the cerebral hemispheres.

The extension of amebic lesions to involve other organs or tissues is reported. For example, involvement of skin in the region of visceral lesions is not uncommon[68,74]. Amebae have been recovered from urine[3,94,98]; penile ulcers[89], and testis and epididymis[101]. Amebae have been found in the leukorrheal discharge of female patients of the Outpatient Department of the National Medical College of Calcutta in a number of cases. Chatterjee[19] notes that various authors have claimed to find the trophozoites of *Entameba histolytica* in practically every organ and tissue of the human body. In some of these, the parasites have been held responsible by various authors for causing inflammatory manifestations such as bronchitis without lung abscess, nephritis, cystitis and urethritis. Amebae have also been discovered in ovarian abscess, salpingitis, vaginitis, in abscess of kidneys and suprarenals and even from lymph glands and bone

marrow of Hodgkin's disease[60,61]. Chatterjee comments that in many cases the observations were neither confirmed, nor were definite proofs as to the amebic nature of the lesions adduced.

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PREVENTIVE MEDICINE AND EPIDEMIOLOGY

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ENDEMIC AND EPIDEMIC DIARRHEAL DISEASE IN ARCTIC GREENLAND

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FIVE years ago this series of epidemiological reviews presented an analysis of the arctic environment and the relation of arctic living to disease in man[3]. In the years since then, three expeditions to the far north have had as their primary purpose field studies on the nature and behavior of acute diarrheal diseases in cold climates. The present report is of observations in Greenland, and a comparison of the findings there with those in other parts of the arctic.

Military operations of World War II brought a new understanding of the arctic and of its significance in world affairs. The extent of war-time activities there is not always appreciated. All political divisions of the far north were involved, those of Russia, the United States, Greenland, Iceland and Scandinavia. Troops of the Allies and of both Germany and Japan took part. Campaigns in the north began soon after the outbreak of war and they lasted until the end of the conflict. As a result, the top of the world acquired an importance that has become increasingly evident in the postwar years: it is the cross-roads between North America and Eurasia.

An expansion of northern populations

is one consequence of the arctic awakening. Continued effort is underway in exploration of the natural resources of the area and their utilization. Trade and travel between temperate regions are directed to advantage over a polar route. All of these developments bring health problems. Immigrants from regions with well-organized health services look to accustomed facilities. Aside from the obligations of an evident need, practical considerations require knowledge of health conditions among the indigenous populations, in line with established military doctrine of the advantages of operating in a healthy environment.

The nature of these health needs is ill-defined, and for a variety of reasons. The cold climate of the north would be expected to modify the behavior of disease, especially the infections which there dominate all others as they do generally in underdeveloped parts of the world. Modes of transmission of infectious agents influence numbers of cases and distributions by time and place. They in turn relate closely to environment, to make necessary an improved understanding of the biological, social and physical attributes of that complex.

The arctic also has its own peculiar diseases and causes of death as do most individual temperate and tropical regions. Diphyllobothriasis is one such condition among infections. Accidental traumatic injury currently holds first place among leading causes of death in Alaska and Greenland. Food supply and nutrition present special problems stemming from terrain, climate and manner of living.

The indigenous inhabitants have an individuality, ethnically and by reason of having had to adapt physiologically and socially to an exacting environment. Newcomers experience the stresses peculiar to transfer to unfamiliar external surroundings, and the differences in this instance are ordinarily great. The host factor is a consideration.

The usual sources of health information are much restricted in the arctic. People are relatively few. They live in scattered villages, sometimes hundreds of miles apart. Appreciable numbers of the native population are seminomadic, incident to seasonal activities in hunting and fishing, the main occupations. As a result, local health services are limited and the reporting of disease highly incomplete. Lack of knowledge about disease frequencies is further exaggerated by extreme difficulties in transport and communication. Extensive epidemics are recorded in the history of the arctic but little is known of the endemic occurrence of those same diseases or of the frequency of more ordinary illnesses where epidemics are not the rule.

Perhaps it is well to interject at this point that the present report deals only with the arctic, that territory within the Arctic Circle and lying north of 66° 30′ North latitude. Conditions are measurably different in the subarctic, still with a decidedly cold climate, but having a population numerically much greater, towns of considerable size, the occasional city, sometimes a developing industry, and a certain amount of agriculture.

The arctic itself is not wholly devoid of these modern developments, in that parts of the Scandinavian arctic by reason of the Gulf Stream are climatically more subarctic than arctic. Found there are the farthest north city in Europe, Hammerfest, population over 5000; the largest city in the world (in area), Kiruna, Sweden, population 18,735; and in Russian Murmansk, population 226,000, a metropolis by most standards and clearly so for the arctic.

In subarctic regions epidemiological information on disease and injury is

ordinarily of a high order, especially in Iceland, the Scandinavian countries and Finland. The same holds relatively for the parts of those countries that extend into the arctic, with the reservation already noted that the Lapland arctic is scarcely arctic in the broad sense of northerly regions as a whole. Much knowledge is to be had from the excellent organizations for health services that exist there, but the arctic, like the tropics and temperate regions, differs so greatly in terrain and populations that broad generalization is not justified.

Multiple civilian and military health problems of a continuing order, the emergencies that arise, and the health services essential to the progress of a developing arctic call for specific and individualized epidemiologic knowledge not now available. Under present conditions the recourse is to field studies. The nature and content of an arctic epidemiology have been outlined in an earlier communication[9a]. As a start toward the objectives defined, preference was for effort centered on one general class of disease processes, with comparative studies of frequency and behavior in representative parts of the far north.

The acute diarrheal diseases have many attractions as material from which to derive principle in an arctic epidemiology. As a group they are essentially universal diseases of man. The recurring variations in numbers of cases is a favorable characteristic. They are established endemically in most populations and commonly at high levels in primitive societies. Epidemics occur with relative frequency and almost everywhere. The observed state of sanitation in the arctic suggests a considerable incidence. General world behavior epidemiologically is well identified through studies that date from the beginning of an interest in community disease. The laboratory support of field studies in these diseases rests on relatively simple methods, a practical consideration under the rigorous conditions of the arctic.

A series of field surveys of acute diarrheal disease, each of about one year, have been made in the three representative parts of the arctic: tundra, taiga and glacier. The first was in the tundra villages of Alaska[9a] (1954-1955), and the second in the forest and farming communities of arctic Lapland: in Finland[10c,d] and in Norway[23] (1956). The final study was in Greenland, in settlements along the west coast of that great island. All four study sites were close to 70° North latitude, about 150 miles above the Arctic Circle. The Greenland investigations now to be presented illustrate general method. Important differences in the physical and social environment of each area are reflected in disease behavior. The results of the several studies will be compared in an effort toward generalization on the nature of acute intestinal disease in the far north.

Organization for Field Study. Even for local activities close to a central or operational base, the success of field studies is influenced greatly by the extent to which cooperation is effectively engendered with local health authorities. In comprehensive investigations of appreciable duration, as in this prospective prevalence survey of acute intestinal disease, more is required than cooperation. The full benefit is from collaboration, to include active participation in the work of the day. This holds still more for operations in a foreign country, where experience dictates that the work easily functions best as a joint undertaking. The interest of local authorities is needed from the earliest stages of planning. From that source comes an invaluable knowledge of local health

conditions, a command of the language, and an understanding of local customs and of the people that are important considerations when the studies are off the beaten track. Perhaps most purposeful is the stimulus given to continued investigation and to a program for action derived from the results of the particular study.

Conferences were held in Copenhagen by representatives of the Government of Denmark, and of the Harvard School of Public Health, Department of Epidemiology. By good fortune they included Dr. Preben Smith, Chief Medical Officer for Greenland, at that time in Denmark on home leave. By mutual agreement the Danish government procured the necessary travel permits, provided access to medical records of the area and enlisted participation of local health and other authorities. The State Serum Institute agreed to act as the base laboratory for the proposed expedition, responsible for the more complicated and specific examinations of material collected in the field and beyond the facilities of a field laboratory. The field laboratory itself was staffed by members of the Institute. Harvard University provided the epidemiological staff, equipment for the field laboratory and the needs for travel and maintenance while in the field.

Choice of Study Site. An equally important principle in field studies, applying particularly to work in a foreign country, is prior reconnaissance in the proposed field of operations. A realistic design of field experiment can not be accomplished without knowledge of local conditions, knowledge which comes from an on-the-spot visit and the information to be had from people directly conversant with the region. A preliminary reconnaissance of West Greenland was made in the summer of 1956. Most of the population is on that side of the huge island. Several study sites were considered, from Thule at latitude 76° N to Godthaab, the capital city of Greenland to the southwest and subarctic. The decision as to a project headquarters was for Egedesminde, at the southerly entrance to Disko Bay. At 69° this little town is at about the same latitude as the previous study sites at Wainwright, Alaska, Inari in Finland, and Mälselv in Norway.

The rocky coastline of the region is indented by several glacial extensions of the ice-cap, and a combination of cold, scant rainfall and minimal soil account for the scrub tundra type of vegetation along the shore of Davis Strait. As the headquarters of Egedesminde Medical District, the town of Egedesminde offered the advantage of existing medical facilities, was able to provide laboratory space in the local hospital and was within a day's travel by boat of numerous smaller settlements where some 7000 Greenlanders live, about one quarter of the native Greenland population. The people are predominantly of Eskimo stock but few are without some European heritage. The town of Egedesminde, with a population of 1100 Greenlanders and 200 Danes, is the trading center of the region, along with considerable fish packing for export. The port is open for navigation from May through November.

Water supply in this area is from surface sources. The town of Egedesminde has a distribution system operative during the four essentially frost-free months, the pipes lying on the surface of the ground and the water drawn from a nearby lake. The smaller settlements scattered up and down the coast depend upon lakes and streams during the summer, and in winter on melted snow and ice. As in Alaskan communities, waste disposal is a problem.

Housing in Egedesminde and the surrounding outposts is somewhat better than that among the Alaskan Eskimos, though below Lapland standards. Only in smaller settlements are the old sod huts found. Igloos certainly exist in the arctic, but not in any of the areas included in these studies. Larger towns have electricity, while domestic and imported coal constitutes the principal fuel. In more isolated settlements the dependence is mainly on peat, tundra brush and driftwood, sometimes from as far away as Siberia.

Intestinal Infection in Greenland. Reporting of intestinal and other disease in Greenland is limited by several circumstances. In the first place, the government health services for medical care are located in the main towns of the area and in connection with local hospitals. The services available to the surrounding outposts and settlements are decidedly irregular. Emergency calls are answered to the best ability; many of these journeys come close to the heroic, and medical staff have lost their lives in winter trips across snow and ice, and the unexpected open water in the frozen surface of Disko Bay. Physicians of the health service make scheduled visits to these little communities, their frequency depending upon work load and the conditions for travel by boat or dogsled.

Greenlanders, like most people, tend to let mild diseases run their course without medical attention. Even if the nature of the illness is apparent, the case may or may not be recorded in the annual reports of the Greenland Health Department[4]. Bacteriologic diagnosis of infectious diseases and consequent accurate identification suffers from the almost total lack of laboratory facilities. Specimens for examination must be sent to Denmark, and from Disko Bay and the country to the north there are no outgoing ships or airplanes between late November and May.

Insofar as intestinal infections are concerned, during the period of 1955 through 1957 only some 200 samples of feces were received by the State Serum Institute in Copenhagen from all of Greenland. Despite difficulties of diagnosis and inadequacies of reporting, monthly reports to the Chief Medical Officer of Greenland contain valuable information for each medical district.

Typhoid fever has been recorded in Greenland for more than a century, all along the west coast, but more frequently in northern than in southern communities. A further significant feature of typhoid fever is that 21 out of 34 reported epidemics started in the autumn. Between 1939 and 1957, cases or epidemics of typhoid fever are mentioned in 10 annual reports. Danish workers who have studied this disease most thoroughly in Greenland, Bertelsen[5], Krog-Lund[17c], and Hrovl[15], were all of the opinion that the primary mode of transmission was person-to-person contact.

Paratyphoid fever has only been recognized during the past two decades. The first laboratory identification was early in World War II. Subsequently several outbreaks have been confirmed by the State Serum Institute as due to *Salmonella paratyphi B*. The early cases[4,6] were identified in southern towns. Only once since 1945 have annual reports from the Chief Medical Officer failed to include paratyphoid fever.

In contrast to salmonella infections, bacillary dysentery appears to be represented in the earliest medical records for Greenland. Descriptions strongly suggesting this disease appear in the journals of Paul and Hans Egede for the years 1737 and 1741. Between 1927 and 1957, eighteen annual reports of the Greenland Health Department

mention either scattered cases of "dysenteria," or major outbreaks diagnosed primarily on clinical grounds. In 1932, *Sh. flexneri* was identified bacteriologically for the first time from Julianehaab. From 1954 to 1956, a slow moving epidemic of confirmed shigellosis involved the town and district of Sukkertoppen. Since 1951 *Sh. sonnei* has been isolated on several occasions and from four west coast communities[4,20].

Most cases of acute intestinal disease are reported to official health agencies as gastroenteritis, etiology unknown. There is reason to believe from clinical reports that a variety of conditions are represented, to include food poisoning, enteritis associated with seasonal dietary changes, perhaps diseases of viral and protozoal etiology, as well as an indeterminate number of cases of typhoid fever, paratyphoid and dysentery. Almost every annual report of the Greenland Health Department records epidemics listed under this nonspecific condition. Cases range from a few to well over 1000. The highest attack rates are among children. Between 1952 and 1956 about 1% of known patients died. For these same years, annual incidence rates for gastroenteritis, based on reported cases, ranged from 10.8 to 52 per 1000 population, the average being 27.7.

Judged by reported cases, the epidemiologic pattern of gastroenteritis is in three broad forms. The first type is that characteristic of point epidemics, with large numbers of cases within a short period of days or weeks. During March, 1954, 500 cases of gastroenteritis were reported among the 1719 people of Julianehaab. The same sort of event happened at Jakobshavn in November, 1954, when 200 recognized cases of gastroenteritis were recorded in a population of 1935.

A second general pattern is that of infectious enteritis building up to high rates over several months. The Sukkertoppen dysentery epidemic to be described later fits this pattern.

Finally, in many communities, usually the larger ones, sporadic cases appear with great consistency, as illustrated by the experience of Egedesminde where, during 1955 and 1956, a few cases were reported each month except two, with rates that ranged from 0.03 per 1000 population per month to 1.8.

The Field Study. As in the Alaskan and Scandinavian surveys already reported[9a,10c,23], work in Greenland centered around studies of endemic prevalence, annual incidence, carrier determinations and the analysis of epidemics. Field observations in Greenland were from mid-June to September 1, 1957; the reported period of greatest prevalence is during the summer months. The technical difficulties of travel and of collecting and transporting stool specimens from outlying settlements are so formidable as essentially to preclude winter observations.

The general plan was to obtain past and present histories of diarrhea and to examine single specimens of feces from a representative sample of native Greenlanders. The first group were residents of the town of Egedesminde. An essentially equal population was chosen from the smaller surrounding communities of the Egedesminde Medical District where living standards are generally poorer. They include outposts and settlements, the outposts having from 100 to 300 inhabitants and the settlements sometimes no more than 20 or so. As in the previous arctic surveys[10a,b] the population was studied on a family basis to insure even distribution of both sexes and all age groups.

As field operations began, all doctors and nurses in the Disko Bay area were asked to report cases of gastroenteritis coming to their attention and to sub-

mit fecal samples from such cases. This was to determine the general nature of endemic clinical disease, in supplement to the presumably more severe illnesses of patients admitted to hospital.

A description of the proposed household study and its purposes was mimeographed in Greenlandic and left with the midwives of outposts who are responsible for ordinary medical care in those communities. A similar pamphlet was distributed to each household just prior to family visits. A cordial and sympathetic statement by the Egedesminde Village Council encouraged community participation in the town.

Other than the authors, members of the staff included Anna Pedersen, a bacteriologist from the State Serum Institute of Copenhagen, to whom special obligations are acknowledged, a Danish public health nurse long resident in Greenland and experienced in the ways of the country, and a Greenlander schoolteacher as interpreter. The three medical officers of the state health service regularly stationed at Egedesminde, Dr. Esben Olsen, Dr. Bent Møller-Christiansen, and Dr. Jan Nannestad, from their solid experience gave much practical help in planning field operations. They contributed clinical information on patients in the hospital. One or other had a part in all field visits, shared their transportation by way of the stout little boat, the *Bjarnov,* and provided a knowledge of the country and the people so essential to sound accomplishment.

American members of the staff left Norfolk in late May, 1957, by the first supply ship of the season, along with laboratory equipment and the necessary field supplies. By agreement, contact was made on June 9 with the Danish colleagues coming by air from Copenhagen, at Sondrestrom Air Force Base in southwest Greenland. Together the group proceeded down Sondrestrom fjord and along the coast by small icebreaker to Egedesminde, there to join the Greenland members of the project. Household visits began a few days later.

Family folders were completed for Greenlander households, including information on age, sex, occupation, health status, history of recent illness, and diarrheal attacks during the preceding year, with dates. Specimen bottles containing 25% glycerine in saline were left with each family member for collection of feces to be examined bacteriologically. A container with merthiolate-formalin mixture was for a second fecal specimen intended for identification of intestinal parasites.

Laboratory Procedures. Through efforts of the Egedesminde medical officers, a newly constructed field laboratory was ready and waiting in a wing of the hospital when staff and equipment arrived from overseas. Working space was ample along with such luxuries as running water and electricity. For the intended purpose, the facilities matched those to be found in Boston or Copenhagen; and as for atmosphere, beyond the picture window and past the hospital laundry-line a never-ending parade of icebergs drifted down Disko Bay.

Bacteriologic and serologic examinations in the field were limited to the isolation and broad identification of pathogens. Subcultures were sent to the State Serum Institute of Copenhagen for confirmation and typing. Field methods followed the Kauffman schema[16]. A rather large inoculum of feces was plated on S.S. agar[8]. Another part of the sample (about 1 gm.) was seeded into selenite F enrichment broth[18]. Following incubation for 18 to 24 hours, subcultures were made from selenite broth to a second S.S. agar plate. All plates were inspected

after incubation of about 24 hours. When growth was poor, incubation was continued for another 24 hours. Suspicious colonies were transferred to triple sugar agar[12] and slide agglutination tests were made with diagnostic salmonella and shigella serums. Disposable plastic Petri dishes were used throughout; they proved to be especially satisfactory in field practice.

Incidence of Acute Diarrheal Disease, by Survey. Some 994 people, corresponding to about 90% of the indigenous residents of the town of Egedesminde, were interviewed in the course of the field investigations, Table 1. They were asked about episodes of acute diarrheal disease during the preceding 12 months. The minimum criteria for a recalled case were at least three loose watery stools within a 24-hour period.

TABLE 1.—ACUTE DIARRHEAL DISEASE AS DETERMINED BY HISTORY OF ATTACK THROUGH FIELD SURVEY; CASES OF THE PRECEDING YEAR, INCIDENCE PER 1000 POPULATION PER YEAR AND PREVALENCE PER 1000 POPULATION, BY AGE, EGEDESMINDE TOWN AND 11 OUTPOSTS, WEST GREENLAND, 1957.

	Persons interviewed		*Recalled cases of diarrhea during year preceding interview*		*Incidence of diarrhea per 1000 population per year*		*Cases of diarrhea observed during household visits*		*Prevalence of diarrhea per 1000 population*	
Age in years	*Town*	*Outposts*	*Town*	*Outposts*	*Town*	*Outposts*	*Town*	*Outposts*	*Town*	*Outposts*
0–4	207	220	75	165	362	750	4	12	19	55
5–14	257	285	70	141	272	495	3	3	12	11
15–45	398	426	221	322	556	756	4	3	10	7
45+	125	156	84	104	672	667	1	0	8	0
Unknown	7	10	2	0	286	0	0	0	0	0
Subtotal	994	1097	452	732	455	667	12	18	12	16
Total	2091		1184		566		30		14	

The average annual incidence for Egedesminde residents was 455 cases of diarrhea per 1000 population per year. The data are judged to be fairly accurate for recent weeks, and to have reasonable exactness for the month immediately preceding the household visit. Field experience has demonstrated the decreasing reliability of information obtained by history of attack as the interval increases between the event and the time of interview. This is especially true for illnesses of short duration and of minor consequence in terms of death or persisting disability, conditions such as diarrheal disease, upper respiratory infections or accidental injury. The annual rates cited are therefore presumably less than actual fact. They serve usefully as a means for comparison of frequencies of acute diarrheal disease determined in other parts of the arctic. Data collected by similar procedures and at similar times of the year gave rates of 205 per 1000 population per year in Alaska, 130 in Finnish Lapland, and 194 in arctic Norway[9b], the latter exaggerated by the presence of a mild epidemic during the summer observations were made.

A more reliable estimate of frequency is to be had from information pertaining to the one month immediately preceding the household visit. The rates thus obtained, Table 2, give an annual incidence for the town of Egedesminde of 1207 cases per 1000 population per year. The observed prevalence rates lend support to this figure.

The values obtained are still likely lower than actual annual incidence.

Epidemics often occur in autumn and winter, to give maximum rates for the year in the months of January, February and March. Eliminating the high rates of certain years incident to epidemics, endemic occurrence is relatively consistent in all months as demonstrated by official reports to the Egedesminde District Medical Service, Table 3. The calculated rates of annual incidence just cited are based on information collected during the summer months of June, July and August, at a time of no epidemics and a rather regular occurrence of the disease. Seemingly they are representative of endemic behavior and somewhat less than average total annual incidence as augmented by epidemics, which experience demonstrates as rather certain to occur.

Young children aged 0 to 4 years had the highest attack rates of any age group, as judged by the more reliable data of Table 2; the rates are high when cases recalled during the preceding year are considered, Table 1. A finding that differed from observations in the Alaskan and Scandinavian arctic was the relatively low rates for older children of 5 to 14 years. In those regions the rates for older children did not differ greatly from frequencies in the younger groups. Acute diarrheal disease was also more frequent for adults of Greenland and older persons

TABLE 2.—CALCULATED ANNUAL INCIDENCE OF ACUTE DIARRHEAL DISEASE BASED ON RECALLED CASES OF THE MONTH IMMEDIATELY PRECEDING HOUSEHOLD VISIT, EGEDESMINDE, GREENLAND, AND 11 OUTPOSTS, SUMMER, 1957.

	Persons interviewed		*Diarrhea cases during month preceding interview*		*Calculated annual incidence of diarrhea per 1000 population per year*	
Age in years	*Town*	*Outposts*	*Town*	*Outposts*	*Town*	*Outposts*
0–4	207	220	26	29	1507	1582
5–14	257	285	11	21	514	883
15–45	398	426	50	25	1507	698
45+	125	156	12	18	1152	1384
Unknown	7	10	1	0	1716	0
Subtotal	994	1097	100	93	1207	1016
Total	2091		193		1109	

TABLE 3.—INCIDENCE OF ACUTE DIARRHEAL DISEASE, OFFICIALLY REPORTED CASES PER 1000 POPULATION, BY MONTHS AND YEARS, DISTRICT OF EGEDESMINDE, GREENLAND, 1954 TO 1957.

Month	*1954 Population 2820*	*1955 Population 3140*	*1956 Population 3276*	*1957 Population 3400*	*Average 1954–57*
January	1.1	1.0	4.6	2.6	2.3
February	0.4	0.0	17.1	3.5	5.3
March	0.0	1.0	16.8	1.8	4.9
April	1.4	0.0	1.8	2.1	1.3
May	1.4	0.6	1.1	2.9	1.5
June	1.8	1.9	0.6	1.8	1.5
July	2.5	1.6	4.0	2.4	2.6
August	0.0	1.0	2.4		1.1
September	4.3	4.1	0.9		3.1
October	0.4	6.4	1.5		2.8
November	1.4	1.9	1.3		1.5
December	0.4	1.0	0.9		0.8
Total year	15.1	20.5	53.0	x	28.7

past 45 years than in the other parts of the arctic studied.

The response of the people in the outposts and minor settlements to home visits about diarrheal disease was almost as good as in the town of Egedesminde, where we lived and worked and were well-known. Information and samples of feces were obtained from 1097 persons of eleven communities that ranged in size from 56 to 210 persons. This corresponded to 52% to 92% of residents, and averaged 79%. On the basis of all recalled cases of the preceding year, Table 1, the incidence rates in these outlying populations were greater by close to a half than in the more sophisticated residents of the central town. The more reliable data of Table 2 also gave higher rates at all ages except for adults, where the lesser number of reported cases served to equalize the over-all rates.

The different outposts varied greatly in reported frequency of acute diarrheal disease. For example, one outpost with the imposing name of Quequetarssuatsiuq, meaning "middle sized island," came up with an annual incidence of 1038 per 1000 population for recalled cases of a year, essentially twice the average. The comparable figure for the outpost of Nivaq at the other end of the scale was 300 per 1000. While completeness of reporting is obviously a factor in these variations, differences in rates are to be expected because of limited contact between outposts and their individual isolation. Each tends to develop its own morbidity pattern, often dependent upon chance introduction of an infectious agent.

Prevalence of Acute Diarrheal Disease. The prevalence rates of 12 per 1000 population for Egedesminde town and 16 for the outposts, Table 1, represent active cases of acute diarrhea observed during household visits. The average of 14 exceeds that observed in other arctic regions in about the same proportion as do incidence rates. The prevalence rate in Norway, for instance, was 8. The higher rates for children aged less than 5 years are in accord with incidence findings. Differences between Egedesminde and the 11 outposts are most marked at the extremes of life, although numbers of cases are too small to permit valid conclusions.

As with incidence findings, there was marked variation in rates between outposts. In 6 of the 11 outlying communities, no active cases of diarrhea were observed. Two of the remaining outposts had prevalence rates approaching 48 per 1000 population. Three of the 30 patients with clinical gastroenteritis during the survey had *S. paratyphi B* infection. Two were adults from outposts, and the third was a child in Egedesminde. Cultures of feces from the remainder failed to reveal identifiable pathogens.

Carrier Rates. Bacteriological culture of single specimens of feces from 1840 apparently healthy persons in the Disko Bay area resulted in identification of 6 carriers of intestinal pathogens. A carrier of *Sh. sonnei* was demonstrated among 151 patients admitted to Egedesminde hospital for conditions other than intestinal disorders. Of the 7 identified carriers, 4 harbored *S. paratyphi B* and 3 were carriers of *Sh. sonnei,* to give an over-all carrier rate of 3.5 per thousand population. Only carriers of the paratyphoid bacillus were identified in the general population of the town: 2 of the carriers in the outposts were of *Sh. sonnei;* numbers of persons examined in the two groups were essentially the same. All age groups and both sexes were represented.

Comparable summer carrier rates in

Alaska were higher (40 per thousand) than in Greenland (3.5 per thousand), although the frequency of clinical diarrhea in Alaska was only about half that of Greenland. No bacterial carriers were demonstrated among 879 residents of Finnish Lapland, or 1051 persons in arctic Norway, where acute diarrheal disease is at a measurably lower level.

The carrier rates parallel environmental conditions. The high rates in Alaska and Greenland are in populations concentrated in villages under crowded living conditions and poor environmental sanitation, with conditions poorer in Alaska than in Greenland. The infrequency of carriers in Lapland fits with the general dispersal of the population in individual homesteads and a level of sanitation comparable to that in the northern states of the American midwest.

Animal Reservoirs of Intestinal Infection of Man. The population of dogs in these Greenland communities approximates that of people; for 136 surveyed households in Egedesminde, the count was 854 people and 714 dogs. The close association of man and the sled dog suggests these animals as possible sources of human infection. The dog is also the principal scavenger of human feces. The failure in this experience to identify in man salmonellosis attributable to strains of animal origin is against such occurrence. Human carriers were found in our studies in Alaska but they were rare. Dogs have been demonstrated as carriers of salmonellae[1]; recent studies in Barrow, Alaska[7,11] showed 36 among 240 people and 6 among 86 dogs to be carriers of S. *typhimurium* but no clinincal disease of man was recognized.

Examination of fresh feces from 109 dogs in the Greenland area by methods used for human feces failed to demonstrate salmonella or other possible pathogens. Similar studies in Alaska gave the same negative result. The evidence is against dogs being an important reservoir of salmonellosis in the arctic, at least under endemic conditions.

Endemic Diarrheal Disease. Clinical descriptions and samples of feces from sporadic cases of acute diarrhea obtained through the courtesy of local physicians, and cases encountered during field survey, provided material in search of specific infectious agents involved in endemic disease. Of the 49 case histories and stool specimens collected by physicians, one illness was demonstrated as due to S. *paratyphi B*. The remainder revealed none of the ordinary bacterial pathogens. With due allowance for delays in transport under arctic conditions and likely inadequacies in collection of feces, this was a decidedly low yield. The better conditions for investigation attending cases discovered in field surveys gave somewhat better results, 3 cases of salmonellosis among 30 instances of acute gastroenteritis, but still a relatively low proportion.

This experience was duplicated in other studies of the series. In Alaska, positive findings were of much the same order, with the difference that the agents implicated were shigellae. In Finland, 6% of 326 sporadic cases of acute diarrheal disease were recognized as due to established intestinal pathogens, 11 instances of shigellosis, one of typhoid fever and 7 of paratyphoid B infection. Neither shigellosis nor salmonellosis was noted among clinical cases of a smaller series in Norway. This situation is not unique to the arctic.

In similar studies in Guatemala, Scrimshaw and associates[21] found the proportion of cases of endemic diarrheal disease with a recognized bacterial pathogen to range between 10%

and 15%, primarily shigellae. Epidemics many times have been clearly related to salmonella and shigella infection, to include Alaska and Greenland within this experience. In still other localities[13] shigellosis had dominated the endemic situation. In consideration of causality of acute gastroenteritis universally, the suggestion is for more attention to virus infections, nutritional factors and other possible causes.

Epidemic Intestinal Disease. An outbreak of gastroenteritis occurred in the Egedesminde District among a group of Danish construction workers who arrived in Egedesminde in early May, 1957. The company of 45 men was housed in five barracks, each with 9 or 10 men. Their meals were provided at the company canteen. Each barracks had its own washroom and privy. Acute diarrheal disease appeared on June 16. From then until July 5 the infection spread through that barracks and the barracks immediately adjoining. Five of the 9 occupants in the first barracks had vomiting, diarrhea, tenesmus, and fever. *S. paratyphi B.* was cultured from 3 patients. In the second barracks, 3 persons became ill, with one confirmed as salmonellosis of the same type. Neither cases nor carriers were identified among workers housed in the other 3 barracks nor among employees of the company canteen. The slow continued progression of cases over a 3-week period and lack of evidence incriminating food or water, led to interpretation that person-to-person contact was the probable mode of transmission. The first patient of the outbreak gave a history of a one-day attack of abdominal pain and diarrhea a month prior to the onset of the epidemic, shortly after arriving from Denmark. Any relation of this episode to the subsequent outbreak remains obscure. Cultures were not made during the first attack.

Another epidemic of diarrheal disease was observed during the late summer of 1956 in the outpost of Sarqaq, north of the Egedesminde Medical District. In the course of travels by boat around Disko Bay as a part of reconnaissance preceding these studies, repeated rumors in several of the settlements visited told of trouble on the island of Sarqaq. It was visited in late August, 1956. The outbreak was a reality, but was subsiding at that time. Cases had begun a month or more previously and in the intervening weeks had involved some 132 of the 220 inhabitants of the outpost, both children and adults. Cases were characterized by slight fever, tenesmus, and moderate diarrhea. Bloody stools had been noted in numerous cases, and two were seen personally. Duration of illness was about a week, and only 10 persons were sufficiently ill to require treatment in bed. The epidemic had built up gradually, with no regular progression by continuity. Instead, cases appeared irregularly in various parts of the village. All indications pointed to transmission by person-to-person contact. Laboratory facilities were not available, but clinically the disease was considered to be shigellosis. From official reports, a scattering of 15 more cases appeared through September and October, 1956.

The island of Sarqaq was in further difficulties the following year. In July, 1957, the laboratory at Egedesminde received four samples of feces from Sarqaq adults. Three were from persons with a history of prolonged intestinal illness, and one from a recent case of acute diarrhea. Of 4 fecal specimens, 2 yielded *S. paratyphi B*, one of them from the recently ill person. In view of these findings, the outpost of Sarqaq was visited on July 18.

Histories were taken from 194 people, about 90% of the total popula-

tion. Only some 12% remembered the outbreak of the previous summer which had involved close to two-thirds of the population, an interesting commentary on the reliability of retrospective histories of acute intestinal illness. In the present instance, diarrhea appeared to have been widespread in the community since June of 1957; 66 persons reported at least one episode.

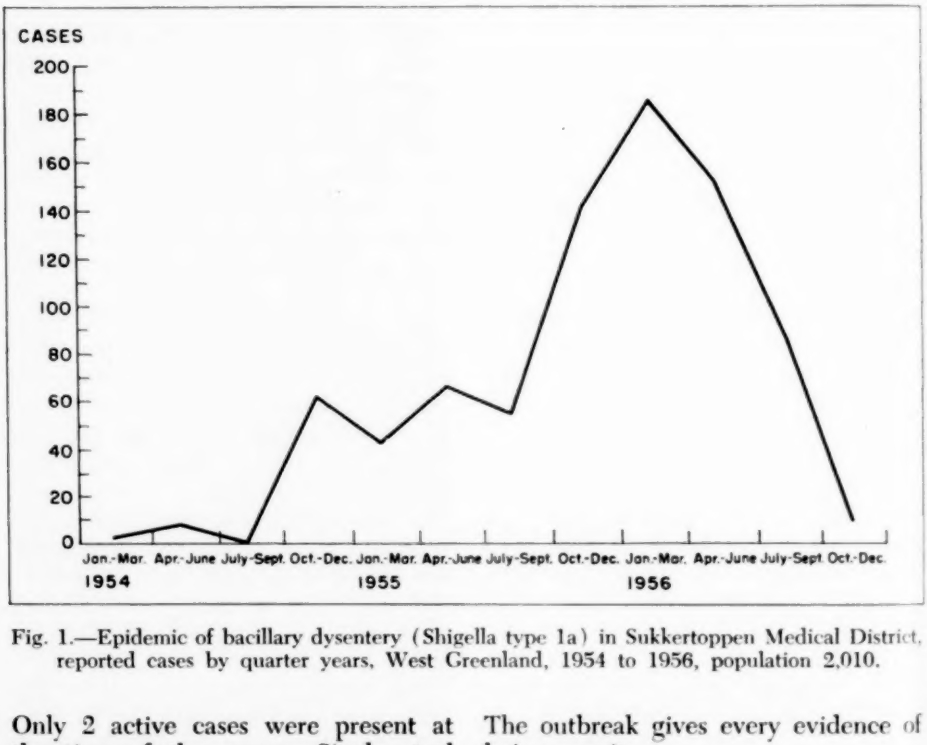

Fig. 1.—Epidemic of bacillary dysentery (Shigella type 1a) in Sukkertoppen Medical District, reported cases by quarter years, West Greenland, 1954 to 1956, population 2,010.

Only 2 active cases were present at the time of the survey. Single stool specimens were collected from 164 people. One more adult and a 5-year-old child were found to be carriers of S. *paratyphi B*. The total carrier rate at the time was thus 2.4%.

The fourth epidemic to be reported began at Sukkertoppen before field studies started. Figure 1 presents the time sequence of reported cases. The data are from monthly reports to the Medical Officer for Greenland[19], abstracted from original reports in the office at Godthaab. During 1954 the disease was listed as gastroenteritis, while cases of the first 4 months of 1955 were reported as dysentery. Between May, 1955, and July, 1956, both designations are used with about equal frequency. During the year July, 1956, to July, 1957, only gastroenteritis appears in Sukkertoppen monthly reports. The outbreak gives every evidence of being a unit.

The gastroenteritis of autumn 1954 was mild, usually without fever, and involved all age groups. Toward the beginning of 1955, cases of a more severe nature were largely restricted to infants and preschool children in a population of about 2200. Fever was clear-cut and loose or watery stools often contained blood and mucus. The disease as manifested in the town itself was not particularly severe, but during

1955 two deaths occurred in children under 4 years residing in the outposts where living conditions are poor and medical treatment not so readily available. Before the epidemic finally subsided, nearly all young children in Sukkertoppen and its outposts were affected.

Examinations of the water supply in the various communities involved were made in attempt to determine the spread of the disease, but no association could be established. The protracted nature of the outbreak, and the irregular and sporadic appearance of cases, speaks for person-to-person transmission; it took a year and a half to involve the whole Sukkertoppen Medical District, Fig. 1. That an epidemic of this magnitude did not spread to other West Greenland communities again illustrates the relative isolation which characterizes settlements of the region.

A diagnosis of dysentery was made by the local medical officer based mainly on clinical behavior of the illness, but supported in part by observations made within the limited facilities of the little hospital laboratory where strains of suspicious bacilli were found in 9 or 10 cases. In 1955 and again in 1956 several pairs of serums from acute and convalescent stages of the disease were tested at the State Serum Institute in Copenhagen and the disease confirmed as due to *Sh flexneri 1a*.

Parasitic Infections. The first comprehensive survey of human intestinal parasites in arctic Greenland[2] gave results more remarkable for the similarities than the differences evidenced as compared with the behavior of helminths and protozoa in other parts of the world. By single stool examination of 663 persons, parasitic infection, all forms, was at a rate of 72 per 100 persons. Prevalence was greater in the more primitive outposts, 76%, than in the town of Egedesminde where the rate was 60%. These geographic differences held at all ages and for all parasites. Children aged 5 to 14 years had the highest rates, then preschool children. Prevalence among adults decreased progressively with age. Rates were continually higher for males than females at all ages, and in the outposts as compared with the town. More persons had multiple than single infections, a finding especially prominent in the outpost population and among children.

Intestinal protozoa outnumbered helminths in a proportion of about 10 to one. *Enterobius vermicularis* was the only common nematode identified. The survey failed to detect a single infection with *Ascaris lumbricoides*, an observation that characterized all of the other arctic areas studied; nor was there hookworm. Presumably these infections are unable to establish themselves because of the ecologic reasons of scanty soil and other conditions favoring embryonation of the eggs. *Metorchis conjunctus*, identified by eggs, was an infrequent parasite in both town and outpost populations; it has been observed in man on a few occasions in Labrador. An unexpected finding was the relatively frequent occurrence (9%) of a trematode tentatively identified as *Cryptocotyle lingua*, a parasite of birds and mammals, not previously described in man. Older adults were the common host and it was found only in the outpost populations.

Entameba histolytica was present in 16% of persons examined, most frequently among children aged 5 to 14 years and in the outposts. The frequency of infection is as high or higher than in many parts of the tropics. In spite of the high infection rate, amebic dysentery apparently does not have an important part in endemic diarrhea in Greenland.

A prevalence of parasites, all forms, of 72%, is decidedly high and comparable to that of many tropical regions. The observation appears reasonable because of the similarity of the arctic and the tropics in primitive housing, crowding within dwellings and highly inadequate sanitary practices. In fact, the microclimate within houses in the arctic commonly comes close to being tropical.

Transmission of Intestinal Infections in Greenland. Acute gastroenteritis as a health problem is neither new in Greenland nor restricted to one locality. This group of diseases is reported regularly year in and year out from settlements with the larger populations and the better medical services. No area in Greenland is consistently free[4]. The bacterial pathogens recognized in Greenland are those found repeatedly in temperate, tropical, and other arctic areas.

Extensive prior contact with members of the salmonella group is indicated by a history of almost annual outbreaks in arctic Greenland generally. The high attack rates among children in epidemics of bacillary dysentery as in Sukkertoppen suggest some previous experience with shigella on the part of adults. Years or even decades may intervene between outbreaks among people living in the isolated settlements which characterize Greenland. Thus, a large fraction of the population in any single locality is likely to be susceptible when a particular pathogen is newly introduced.

The environment has a peculiar attraction in efforts to determine modes of transmission of enteric infection in Greenland. From the standpoint of geography, rocky terrain plus permafrost characterize the coastal area. Water supply is thus restricted to surface sources with the attendant dangers of pollution at the point of origin or along a distribution system, whether the distribution system be a community pipeline or a family bucket. Waterborne infection is a potential danger, but detailed studies in the outbreaks encountered failed to incriminate water as the responsible vehicle. Almost without exception, bacteriological examination of commonly used supplies demonstrated high grade contamination. The protection against involvement in epidemics probably lies in the custom and necessity of each family being responsible for its own needs, with no prevailing common source.

Among 136 households surveyed in Egedesminde, only 28 had a chemical toilet or other indoor provision for collection of human excreta. Nine families boasted an outdoor privy used exclusively by members of that household. The commode is usual. The customary disposal of human wastes is by emptying the contents of commode or chemical toilet on the ground adjacent to the dwelling or into a stagnant pond not far away. During summer, sled dogs run free, consuming garbage and human excreta promptly as discarded from the household. In winter while the dogs are working, the repeated blanketing by snow and the intense cold take care of things effectively.

Where it exists, the Greenland privy is a unique structure. Because of the terrain, it rests of necessity on the ground with no underlying pit. From beneath the seat, a broad shallow chute extends out beyond the back wall of the privy. When a Greenlander enters and bangs the door, dogs gather from all directions to wait at the end of the chute, ready to carry out their function of arctic scavenger. For all practical purposes there is no subsurface disposal of human excreta in most Greenland communities. The implications in spread of intestinal infection are obvious.

Because of the unique canine scavenging function, Krog-Lund[17a] undertook experiments in feeding cultures of typhoid bacilli and stools of human carriers to dogs. While he did not establish these animals as reservoirs of infection, he did demonstrate that sled dogs may excrete viable members of the salmonella group recurrently and for short periods.

Even to a greater extent than in Alaska or Scandinavia, vast expanses of land in arctic Greenland are without human habitation, covered by glacier and rarely explored. Life is along the coastal fringe and that mainly to the west. Crowding within villages and dwellings is nevertheless often extreme. The Egedesminde survey showed the average number of persons per house to be more than 6; occasionally as many as 12 live in one or 2 rooms. It is common practice for the entire family to sleep together on a kind of low shelf along one wall. That was the situation in 43 out of 136 Egedesminde houses surveyed; it is even more frequent in the smaller outposts where living standards are closer to those of the Alaskan Eskimo. When precious fuel must be used to melt snow and ice, or water has to be carried long distances, it is not surprising that this commodity is used sparingly. Hollister[14] and associates have established the influence of water availability on shigella prevalence.

Poor sanitation, scarcity of water, and overcrowding are all factors favoring the spread of disease by person-to-person contact. The outbreaks reported in the present studies seemed by their epidemiologic behavior to have been propagated in this manner. They were characteristic of many epidemics of gastroenteritis in the past.

Bertelsen[5] has stated that "typhoid fever in Greenland as a rule spreads directly from man to man." Likewise Krog-Lund[17b] postulated for the same disease that the most important means of spread is by "very massive ingestion of more or less fresh bacillary particles from carriers' hands, nails, underwear, etc." Commenting on 3 epidemics, he noted that all had an insidious beginning, cases were limited to specific families and acquaintances, and the epidemics were protracted in time.

The same mode of transmission has been advanced for dysentery. Writing about an outbreak of that disease in Godthaab, Børresen (1931) is quoted by Bertelsen[5] as stating that "feces is probably the common source of infection. Drinking water and food hardly play any role in this respect." The accumulated evidence suggests that contact transmission is the primary means by which typhoid, paratyphoid and dysentery spread in Greenland, endemically and in epidemic proportions.

In supporting this conclusion, unprotected water supplies and food as a vehicle are not to be eliminated as sources of infection in sporadic cases or even family outbreaks. In other investigations of this series, a small outbreak of staphylococcal food poisoning was observed in a camp in Alaska and another in a residential school in Lapland. During summer, refrigeration of food is minimal or nonexistent even in the better houses, but fortunately temperatures are seldom sufficiently high to promote rapid multiplication of infectious agents.

Food-borne epidemics of the common diarrheas and dysenteries are rendered unlikely because each family tends to provide its own food supply through the individual skill of the hunter of the group. No such epidemic of consequence has been reported. However, the outbreak of trichinosis[22] due to walrus meat remains a classic in arctic epidemiology and a further potential exists in the liking of the Greenlander for social gatherings with food a main attraction.

Certain foods come into common use, notably whale meat during summer while there is an open sea. These great sea mammals are captured by a small trawler, butchered, and the meat sold from the ship and from settlement to settlement, uncovered, unrefrigerated, walked on by crew members and suffering manifold sanitary indignities.

Summary. During the summer of 1957, field studies of acute diarrheal disease in the Disko Bay region of West Greenland demonstrated an average annual incidence of 1109 cases per 1000 population, based on cases of the preceding month reported during household interviews of 2091 people. Prevalence rates, existing cases on the day of investigation, averaged 14 per 1000 population. The age group 0 to 4 years was most often affected.

Carrier studies, based on single stool examinations of persons without diarrheal disease revealed 4 individuals harboring *S. paratyphi B* and 3 carrying *Sh. sonnei*. The carrier rate was 3.5 per thousand population, and all age groups were represented.

Epidemics of paratyphoid fever and of bacillary dysentery were encountered. They tended to be relatively extensive, to evolute slowly and to be of long duration. Person-to-person contact, secondary to features of the physical and social environment, was the apparent mode of transmission.

The frequency of intestinal parasites rivaled that of the tropics, 72%. Although 16% of the surveyed population were found to harbor *Entameba histolytica*, this infectious agent was apparently responsible for little of the prevailing diarrheas and dysenteries.

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PHYSIOLOGY

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SENSORY PHYSIOLOGY

SESSION OF FEBRUARY 21, 1961

Combined Functions of Rods and Cones. JOHN LOTT BROWN, PH.D. (Department of Physiology, University of Pennsylvania School of Medicine). The time course of dark adaptation was measured following light adaptation to a 9000 ft.-lambert field for a period of 3 minutes. Measurements were made for each of two observers and with test light wavelengths of 464 and 564 millimicrons. Three criteria of threshold were employed: detection of light, and recognition of the orientation of each of two grating patterns which required visual acuities of 0.05 and 0.07 for their resolution. Threshold measurements were made in a circular region of the retina 2½° in diameter and centered at a point 7° from the fovea. Dark adaptation curves presented on a logarithmic luminance scale based on a scotopic standard of luminosity all have two clearly defined branches. Early branches are all of the same form, but displaced along the log luminance axis for different test flash wavelengths. Secondary branches are of the same form and superimposed for curves which represent visual acuity criteria, but differ in form for the two test wavelengths when the criterion of threshold is light detection. Variations in shape and position of the dark adaptation curves with changes in test flash wavelength suggest that rods may be making some contribution to the initial branches and that cones may be making some contribution to secondary branches. There is also a possibility that the scotopic spectral luminosity standard which was employed may actually apply to a mixed population of rods and cones rather than to rods alone. The results illustrate the importance of threshold criterion and spectral character of the illuminant as well as adaptation in the determination of the receptor population which subserves visual performance. It is clear that simple dependence on standards of luminosity for the estimation of luminance requirements is not sufficient in many situations in which a high order of precision may be desired.

Limitation of Ocular Motility in Humans Undergoing Positive Acceleration (Loma Test). E. L. BECKMAN, CAPTAIN, MC, USN, K. R. COBURN, LCDR, MSC, USN, AND T. D. DUANE, M.D., PH.D. (Aviation Medical Acceleration Laboratory, U.S. Naval Air Development Center, Johnsville, Pennsylvania). In a search for a reproducible and objective endpoint for measurement of reaction to positive acceleration, it was observed that purposeful ocular motility disappeared at approximately the same magnitude of acceleration that produced a loss of peripheral vision in 154 determinations on 22 subjects. Further studies designed to elucidate the mechanism of this phenomenon were carried out on trained centrifuge subjects and it was disclosed that these subjects could, in fact, rotate their eyes after losing peripheral vision but in an ataxic manner. The opticokinetic nystagmus persisted after

subjective loss of peripheral vision (grayout) and loss of central vision (blackout) although the rate and rhythm of the nystagmus were altered. The ability to follow a moving target was also lost after peripheral vision failed. Progressive pupillary dilatation was observed, beginning at the time that the peripheral vision was lost and becoming maximal after central vision failed. The above phenomena were also tested while 30 to 35 mm. Hg negative pressure was applied over the eyes by use of a modified underwater swimmer's mask. With this technique, vision after blackout during acceleration was restored almost to normal. The purposeful eye movements likewise improved. The opticokinetic nystagmus was restored to control rate and rhythm as was the target following reflex. The pupils, however, remained dilated. On the basis of these findings, it is concluded that there is a cerebral cortical dysfunction which occurs during exposure to positive acceleration at levels which cause blackout. This is in addition to the commonly accepted retinal failure. The mechanism by which the cerebral dysfunction and retinal failure interact to explain the above findings remains to be elucidated and is under study at the present time.

Vestibular Function in Space Flight. R. F. Gray, (M.A.) (Naval Air Development Center, Johnsville, Pennsylvania). The problems of motion perception in orbital space stations centers around the question, "should or should not space stations be rotated to create an artificial gravity for the comfort of its crew?" In an orbital space station gravity is canceled by centrifugal acceleration which exists because of the high speed with which the station rotates around the earth. This cancellation makes the inhabitants of the space station weightless which is a new experience for mankind, since history indicates that no man has yet been exposed to weightless situation for longer than about one minute. Some of the principal organ systems involved in the detection of acceleration of the body, are those in the inner ear; the semicircular canals and the otolith organs. The present paper discusses three methods of reducing the effect of gravity on the otolith organs which are currently being used in weightlessness simulation. These are (1) flights in parabolic arcs, (2) submersion of inverted subjects in water and (3) tumbling around a horizontal axis.

The second problem, discussed in this paper relates to motion sickness effects which occur when persons move around in a rotating environment. Such effects seem to be related to coriolis acceleration, an acceleration which is generated as a result of the aforementioned combination of motions. The background of mechanics and physiology involved in stimulation of the ear by coriolis acceleration are discussed, as are the problems of predicting from studies conducted at the surface of the earth, those sensations which will occur in rotating space stations.

New Concepts of the Role of the Central Nervous System in Hearing. S. D. Erulkar, Ph.D., and P. G. Nelson, M.D. (Department of Pharmacology, University of Pennsylvania Schools of Medicine and National Institutes of Health, Bethesda). Responses to acoustic stimulation of single neurons of the inferior colliculus and medial geniculate body of the cat have been recorded by intracellularly placed microelectrodes. Spike potentials varying in amplitude to 35 mV have been recorded; the responses from the majority of the neurons, however, consisted of excitatory (EPSP) or inhibitory (IPSP) post synaptic potentials or combinations of the two.

Binaural stimulation: EPSPs and IPSPs could be evoked by clicks delivered to either ear, but their magnitudes were dependent upon which ear was stimulated. In some neurons, for instance, in response to a contralateral stimulus, an EPSP would predominate, while for those same neurons, in response to an ipsilateral stimulus, the IPSP would be greater in amplitude. In other neurons, a contralateral click could evoke a pure EPSP, while an ipsilateral click evoked a pure IPSP. In order that the responses to binaural stimulation should interact, a most delicate timing of the arrival of the impulses at the neuron is required.

Tonal frequency: The change in frequency of a tonal stimulus could change the relative amplitudes of EPSPs and IPSPs in a response from a single neuron, the EPSP predominating at certain frequencies, the IPSP at others. When the neuron responded either with a pure EPSP or a pure IPSP, both the amplitude and the time course of the potential changed as the frequency was changed.

Intensity: An increase in the stimulus strength could cause a differential growth of the EPSP and the IPSP of the response of a single neuron.

The inhibition present at inferior colliculus and medial geniculate need not represent merely the withdrawal of excitation at some other level, but can be an active process taking place at the sites of recording. This has been shown by the alteration of the amplitude of postsynaptic potential by polarization of the cell membrane by currents delivered through the micropipette.

BOOK REVIEWS AND NOTICES

HENRY STANLEY PLUMMER: A DIVERSIFIED GENIUS. By FREDRICK A. WILLIUS, M.D., M.S.(IN MED.), Formerly Head, Section on Cardiology, The Mayo Clinic. Emeritus Professor of Medicine, the Mayo Foundation for Medical Education and Research, University of Minnesota, Rochester, Minnesota. Pp. 71; 13 ills. No index. Springfield, Ill.: Charles C Thomas, 1961. Price, $4.50.

THE glamour surrounding the Mayo Clinic has naturally focussed widespread attention on many of the aspects of that famous institution—its methods, its architecture, and the protagonists of its personnel. Seldom has a new institution assembled such a galaxy of surgeons and physicians as the Mayo brothers gathered in the small Minnesota town of Rochester. Always concerned primarily with the patient's welfare, the far-sighted William and Charles led the staff in the prosecution of clinical and laboratory research. One of the latter type was Henry Plummer, son and grandson of physicians, who was born in 1874 in a village near Rochester. It is hard to realize that Rochester itself was but a small town, as is shown by the picture (p. 4) of the deep rutted main street, with ox-drawn Conestogas, taken a few years earlier.

Henry even as a child progressed successfully through local grammar and high schools, and then after 2 years of academic studies at the University of Minnesota and 3 years of medicine at Northwestern (M.D. in 1898), he returned to join his father in practice at Racine, a village near Rochester. Once, a few months later, he had to substitute for his sick father to drive Dr. Will Mayo to see a patient with a puzzling blood disease. Aided by his microscope, which he had taken with him, he demonstrated an aleukemic leukemia so brilliantly that Dr. Mayo promptly added him to the Mayo staff.

He came at a fortunate moment: a brilliant group of younger clinicians was already assembling and Plummer was assigned to expand and modernize the laboratories. At that time little was attempted there more than routine blood and urine tests. X-rays, then in their infancy (only 6 years after Roentgen's discovery), attracted Plummer's interest especially, both for diagnosis and treatment. His first publications (1906-1909) were appropriately on spasm and diverticulation of the esophagus. He was also the first at the Clinic to use the bronchoscope for procuring tissue for diagnosis as well as for removal of foreign bodies and dilation of strictures. His early interest in goitre was renewed, stimulated by the abundant material that had by now become available. With E. C. Kendall, a later Nobel Prize Winner, who had come to the Clinic to head the Section on Biochemistry, numerous studies of value were made; and also with W. M. Boothby, whom he had invited to create a metabolic laboratory. This association lasted till Boothby died. The relation of Kendall's thyroxin to hyper- and hypothyroidism, studied with their new "basal metabolism" method, became the basis for a useful classification of goitres (nontoxic adenomatous, toxic with hyperthyroidism, exophthalmic hyperplastic with an excess of thyroxin deficient in iodine). Later pre-operative iodine treatment was found to diminish the crises that caused such havoc and to lower the mortality after thyroid removal to less than 1%. Electrocardiography, just being introduced into this country, so revealed its potentialities to Plummer that he got the author of this book to develop the subject at the Clinic. Plummer, characteristically, was always at hand to help the younger man conquer the whims of their cranky Cambridge model.

Many other of Plummer's activities could be mentioned to demonstrate the diversity of his genius—his intuitive utilization of psychology in aiding the neurotic; his skill in welding the products of laboratory research to clinical experience; his successful concern with medical education and his teaching methods; his use of mechanics in guiding the building programs and choice of installations in the rapidly expanding medical center; these are some of the chief of his diverse scientific contributions.

His avocations and cultural interests were by no means neglected. He loved good music and classical art. He was an avid reader of literary classics and both general and medical history; his facile, retentive mind thus getting stimuli for worthwhile applications. He has been rightly called, by one who knew him well, a great physician, a master of the science and art of medicine, a teacher surpassed by few, and a learned scientist in many fields. Hunting and fishing, his early sports, eventually gave way to motor cruising with his family and friends through the many lakes and waterways of the surrounding country.

These avocations were not enough, however. In spite of increasing vascular trouble, he continued on the job, spending the last day of his life at work at the Clinic. He drove himself home, where he found he could not walk unaided. Assisted to his bedroom, he summoned his family, giving advice and predicting that within the hour he would become unconscious. This occurred close to the hour, the coma persisting until death ensued. He was 62 years old.

This well written little book, though unaided by any glamour of wide clinical publicity, should ensure that Henry Plummer will be remembered as a leader among the great men at "the Mayos." E.K.

PATHOLOGY: A DYNAMIC INTRODUCTION TO MEDICINE AND SURGERY. By THOMAS MARTIN PEERY, M.D. AND FRANK NELSON MILLER, JR., M.D., Professor of Pathology and Associate Professor of Pathology, The George Washington University School of Medicine, Washington, respectively. Pp. 626. Boston: Little, Brown & Company, 1961. Price, $5.50.

THIS is an excellent textbook of Pathology and being a paper-bound edition it should be a boon to medical students and graduates alike. It has always been my contention that medical textbooks in general have a short lived span and that the expense of having an up-to-date or current library is almost prohibitive for the average individual. However, paper bound copies would overcome this difficulty. Admittedly illustrations or pictures are curtailed or absent, but, as the authors of this particular text state in their foreword, the book can and should be supplemented by gross and microscopic material. The authors are to be congratulated for their concise and clear-cut style; they have been able with the use of a few words to make this text more than a summary or a compendium of pathology. The material is up-to-date and a short key reference list is given at the end of each chapter. The text follows the usual presentation of the subject. There are section headings and marginal notes are inserted beside practically each paragraph indicating their contents. Much of the significant data is underlined. I feel that the authors have accomplished their objective in turning out a manuscript to help the medical student to learn to be a doctor and to see the vision of that goal in his study of pathology and to obtain that goal by his knowledge of pathology. Certainly the medical student and the graduate can understand and appreciate pathology by a study of this particular text. A.P.

FETAL ELECTROCARDIOGRAPHY. THE ELECTRICAL ACTIVITY OF THE FETAL HEART. By SAUL DAVID LARKS, B.S.E.E., M.S.(E.E.), PH.D., Assistant Professor of Biophysics, School of Medicine, The University of California, Los Angeles. Pp. 109; 70 ills. Springfield, Ill.: Charles C Thomas, 1961. Price, $6.50.

IT is a difficult task to obtain a fetal electrocardiogram. Yet, it can be done and demonstrated to provide information not otherwise obtainable. The author is well known for his interest in this subject and is responsible for much of our present knowledge of it. He is its master, and his use of this tool is brilliantly described and demonstrated. Following introductory chapters concerned with relevant historical aspects, embryology and bioelectric phenomena, the technique for obtaining a fetal electrocardiogram and leads used are described. These have allowed for recognition of a human fetus as early as 11 weeks, twins at 12 weeks, and position *in utero*. Fetal distress can be recognized by change in rate, and fetal cardiac abnormality by change in rhythm or QRS complexes. The potentialities of fetal electrocardiography are unlimited, and the author's enthusiasm, generated by meticulous time-consuming work, should be contagious.

J.Z.

PIONEER MICROBIOLOGISTS OF AMERICA. By PAUL F. CLARK, Emeritus Professor of Medical Microbiology, The University of Wisconsin. Pp. 369; 38 ills. Madison: The University of Wisconsin Press, 1961. Price, $6.00.

IN a short book with delightful style, the author has admirably told the story of the United States' first and second generation of microbiologists. He has limited his area to the "bursting infancy and teen age period" of the science in this country which extends up to the first World War. However, some diseases are discussed without restriction to time or country.

The book is organized according to diseases, geographical section of the U.S., or institution. To this framework, are added the appropriate personal sketches of contributors of significant knowledge. Since there are so many sketches, some are quite short; but, through the precise choice of words, the reader is told much about each person, his

accomplishments and his failures. Praise is not overabundant; only Zinsser is called brilliant. The last chapter, Epilogue—From a Laboratory Window, is especially enjoyable.

The book is remarkably free of printing errors, is well indexed, and is well documented with references listed by chapter at the end of the book. It is recommended to students of medicine at all levels. The cost is proper. K.S.

TOBACCO: EXPERIMENTAL AND CLINICAL STUDIES. By P. S. LARSON, PH.D., H. B. HAAG, M.D., AND H. SILVETTE, PH.D., Professors of Pharmacology Medical College of Virginia, Richmond. Pp. 932. Baltimore: The Williams & Wilkins Co., 1961. Price, $20.00.

ALTHOUGH most physicians are fully aware of the disputes that exist concerning the effects of tobacco in the human and other species, it is probable that few are cognizant of the staggering extent of confusion in scientific literature concerning this topic. This monumental review of more than 6000 articles up to and largely into 1959 brings home to the reader the pressing need for far more carefully designed experiments before final answers can be provided to many important questions. There is little consistency of data in any area of observation, and unanimity of opinion is even more rare. Faced with this problem in the preparation of their review, the authors felt that it would be best to be "deliberately non-selective, printing (as it were) good work and bad, sound and uninformed opinion, alike, in the same size type." They leave the reader to draw his own conclusions, but they have helped immeasurably by including much valid criticism and by pointing out the many pitfalls in interpretation. The reviewer found it very valuable to read carefully many sections concerning questions that have vexed him, and, though being amazed by the wide disparity of opinion, the result was a much better understanding, and an opportunity to select a reasonably satisfactory guide for practice. Take, for example, the subject of the type of smoking most often involved in tobacco amblyopia. Here, as elsewhere, there is difference of opinion but it is hard to escape the weight of evidence pointing to the pipe as the prime offender. No doubt many physicians will be interested in the sum total of evidence concerning tobacco and lung cancer and they will have thereby a better opportunity than ever before to judge for themselves (free, I hope, of personal prejudice) the degree of incrimination of the smoking habit.

The volume is designed primarily for the investigator and the specialist in various fields, but it is hoped that the general practitioner will find it a source for reference concerning the questions that constantly are arising in practice of any type. The only wish of the reviewer is that the authors had been more clinically oriented to avoid such absurdities as the inclusion of the effort syndrome along with effort angina. T.D.

CONGENITAL MALFORMATIONS OF THE HEART. VOLUME II. SPECIFIC MALFORMATIONS. By HELEN B. TAUSSIG, M.D., Professor of Pediatrics, Johns Hopkins University. Pp. 1049; profusely illustrated. Cambridge: Harvard University Press for the Commonwealth Fund, 1960. Price, $17.50.

IT is unfortunate that Maude Abbott could not have lived to read this volume. Dr. Abbott devoted her life to the study of congenital heart disease in a day in which the only apparent gain from accurate diagnosis was the possibility of arriving at a somewhat reasonable prognostication for the victims of these malformations. Today the situation is a very different one, and it is highly instructive and refreshing to read this volume by the present high priestess in this disease temple. She is not only extensively experienced in the field, but she is blessed, in addition, with that rare talent, the ability to teach what she knows. Her imaginative approach to therapy is well illustrated by the fact that she was the first to devise a means of approach to the blue baby problem.

Volume I considered the basic methods of approach for diagnosis, plus fundamentals of therapy. The present volume gives detailed information concerning each of the specific malformations. The very lucid diagrams which accompany the discussions are of great value. At the end of the book there is a chapter of visual indices which presents the most concise summarization of significant findings the reviewer has ever seen. Every physician dealing with congenital heart disease could not do better than to read and digest this volume. T.D.

ELECTROLYT-KOMPENDIUM. PHYSIOLOGY, PATHOLOGY AND THERAPY OF WATER AND ELECTROLYTE EQUILIBRIUM. By WERNER FLEISCHER, Department of Pathology, Veterans Administration Hospital, Milwaukee,

Wisconsin, and E. FRÖHLICH, 4th Medical Department, District Hospital, Graz. Pp. 380; 30 ills. Basel: Benno Schwabe & Co., 1960. Price, $9.50.

THIS volume is a fairly complete but brief summary of the general principles, a knowledge of which are necessary for the discussion; the pathophysiology of the various disorders of water balance and electrolyte equilibrium, and the clinical evaluation and therapy of these disorders. The specific clinical problems faced include those caused by pituitary disease, adrenal disorders and various bony changes, as well as those seen in diabetes mellitus and cardiac dysfunction.

This volume is pointed throughout toward clinical application and the thorough review of basic principles affords a firm base of operations for the author and reader alike, and the clinical discussions lean heavily and profitably upon this previous preparation.

J.B.

DUNHAM'S PREMATURE INFANTS. By WILLIAM A. SILVERMAN, M.D., Babies Hospital, New York. 3rd ed. Pp. 578; 72 ills., 113 tables. New York: Paul B. Hoeber, Inc., 1961. Price, $15.00.

THE mantle of responsibility for this new edition of a medical classic has fallen on worthy shoulders. The excellence of the volume deserves the acclaim of all interested readers and particularly those charged with the care of premature babies. A great deal of new information has been included with adequate annotation of recent date. Theoretical and practical aspects of the subject are presented, as well as necessary definitions and statistical directions. Although the style is occasionally brusque and the opportunity for broader discussion often avoided, the compilation is extensive and unsurpassed in its field. The text is liberally complemented by self-explanatory illustrations and tables. It is recommended highly.

A.B.

DOCUMENTA GEIGY WISSENSHAFTLICHE TABELLEN. Edited by K. DIEM. 6th ed. Pp. 742. Basel: J. R. Geigy, 1960.

THIS handbook of scientific tables has been expanded to include much new material. For example, a section authored by H. Krebs, outlines the chemistry and intermediary metabolism of the major foodstuffs. Many of the other similar sections dealing with biological materials have been revised. The tables will be useful chiefly to those in the biological sciences although many of the mathematical tables will have more general application.

To merely illustrate the range of topics included, it must suffice to mention these few: 1) Numerous tables of mathematical and statistical functions. 2) Tables of physical and chemical constants. 3) Outline of the chemistry and intermediary metabolism of the foodstuffs. 4) Chemistry and physiology of the vitamins and hormones. 5) Nutrition. 6) Chemical composition of various organs and biological fluids. 7) Outline of water and electrolyte balance. 8) Various medical and biological normal values. 9) Infectious agents and diseases.

This is a very complete and useful compendium. Most of the tables include careful definitions of the units and factors used. If it were in English instead of German, most biological investigators in this country would want to have it in their library.

J.B.

MULTIPLE-CHOICE EXAMINATIONS IN MEDICINE. A GUIDE FOR EXAMINER AND EXAMINEE. By JOHN P. HUBBARD, M.D., Professor of Public Health and Preventive Medicine, University of Pennsylvania, and WILLIAM V. CLEMANS, PH.D., Director of Testing Services, National Board of Medical Examiners, Philadelphia. Pp. 186. Philadelphia: Lea & Febiger, 1961. Price, $3.75.

THIS excellent volume, published in 1961 by Lea & Febiger of Philadelphia, should be required reading for the medical educator.

The authors, John P. Hubbard, M.D. and William V. Clemans, Ph.D. qualify as authorities through long experience with the National Board of Medical Examiners; Dr. Hubbard as Executive Secretary and Dr. Clemans as Director of Testing Services of the National Board. Dr. Hubbard is also Professor of Public Health and Preventive Medicine, University of Pennsylvania School of Medicine.

In this precise 180 page volume, the advantages and disadvantages of the objective examination are presented and discussed. Comparison is made in analytical fashion with the essay type examination. A chapter containing sample test questions from the National Board is included.

This book is truly "A Guide for Examiner and Examinee."

G.R.

SURGERY OF THE AORTA AND ITS BRANCHES. By JAMES D. HARDY, M.D., Professor and Chairman of the Department of Surgery,

University of Mississippi Medical Center and Surgeon-in-Chief to the University Hospital. Pp. 386; 75 ills. Philadelphia: J. B. Lippincott Company, 1960. Price, $6.50.

THIS book was written primarily to familiarize the physician not involved in the practice of vascular surgery with what can be accomplished by modern surgical techniques. Although it is written in the form of a handbook and does not pretend to go into minute operative details, it is surprisingly complete in its coverage, taking up most if not all of the problems facing the vascular surgeon. Both the diagnostic and therapeutic techniques commonly in use today are described.

While the book is not written for the vascular surgeon, the vascular surgeon may find it helpful because of the exhaustive list of references at the end of each chapter. The book will be found to be useful out of proportion to its size. J.J.

RECENT ADVANCES IN HUMAN GENETICS. Edited by L. S. PENROSE, M.A., M.D., F.R.S., Galton Professor of Eugenics, University College, London. Pp. 194; 13 ills. Boston: Little, Brown & Co., 1961. Price, $8.00.

THIS is a short, up-to-date, well edited treatise which adheres to the area of its title, human genetics. It consists of 8 chapters contributed by American and British authorities. The topics of the chapter are so divergent that repetition is avoided. Two of the papers concerning human chromosomes owe their existence to the 1956 demonstration of Tjio and Levan that the human chromosome count is 46 not 48.

Other areas covered are mutations, developmental sex abnormalities, genetics of growth and development of the fetus, inheritance of finger and palm prints, abnormal hemoglobins, gene order, and statistical methods.

The book should be of interest to physicians concerned with the genetics of disease, obstetricians and pediatricians: it should find a place in medical school courses in genetics. Instructors might be dissuaded from making this a required text, however, because of its price which seems excessive. K.S.

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